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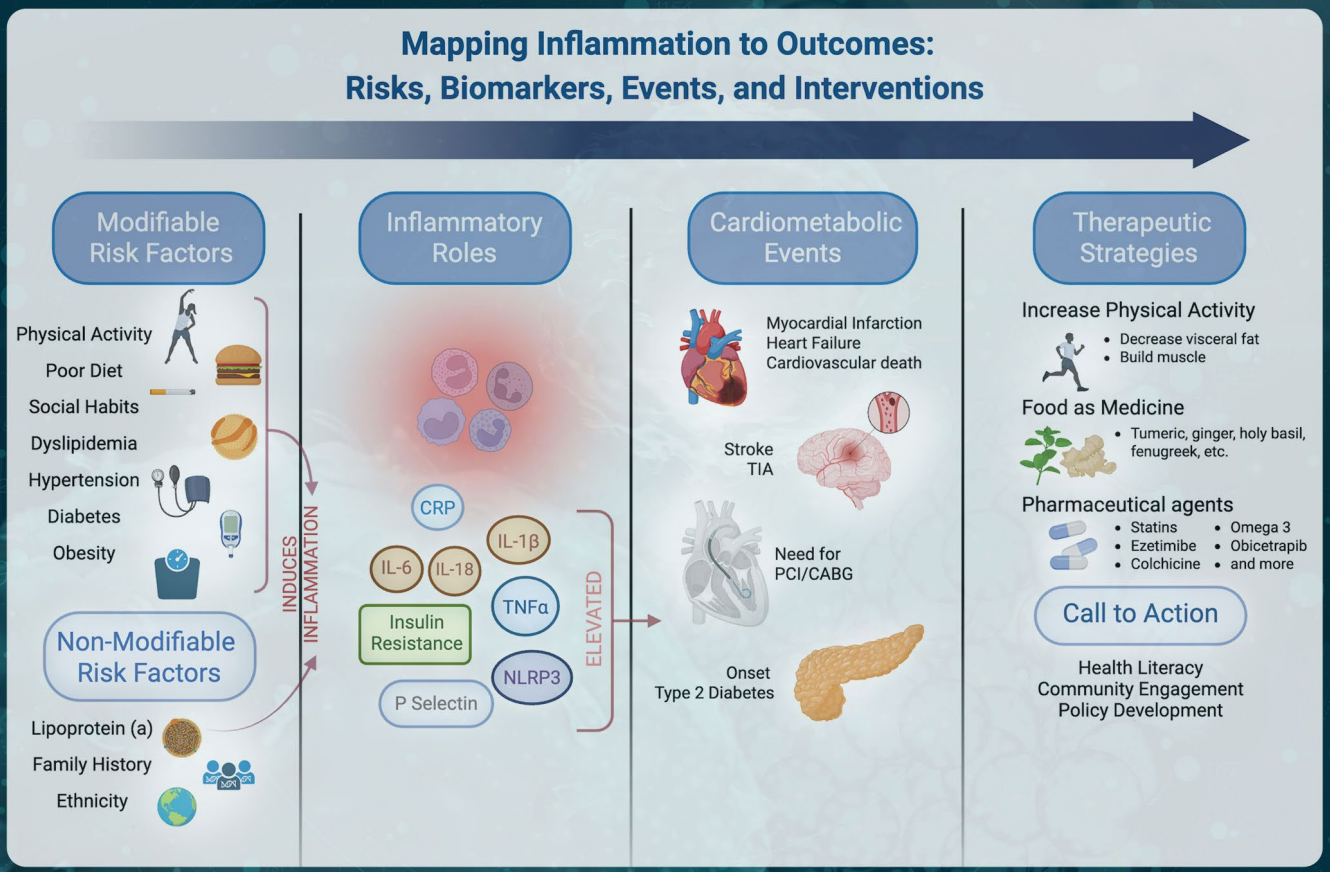


## Inflammation and cardiovascular disease – Part I: Mechanisms and biomarkers

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REVIEW ARTICLE

# Inflammation and cardiovascular disease – Part I: Mechanisms and biomarkers

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## Abstract

Inflammation is a fundamental driver of atherosclerotic cardiovascular disease (ASCVD), orchestrating immune activation, endothelial dysfunction, and plaque instability. While lipid-lowering therapies can reduce the burden of ASCVD, persistent inflammation remains a critical determinant of residual cardiovascular risk, highlighting the need for deeper investigation into inflammatory pathways. Key mediators, including interleukin-6, high-sensitivity C-reactive protein, and myeloperoxidase, amplify immune cell infiltration, foam cell formation, and extracellular matrix degradation, exacerbating atherosclerotic progression. Beyond these well-established markers, emerging inflammatory biomarkers, such as cluster of differentiation (CD)47, serum and glucocorticoid-regulated kinase 1 (SGK1), P-selectin, and growth differentiation factor 15 (GDF15), provide novel insights into vascular inflammation and immune dysregulation. CD47 modulates macrophage-mediated immune evasion, allowing apoptotic debris to accumulate within plaques, while SGK1 enhances pro-inflammatory signaling and endothelial dysfunction. P-selectin facilitates leukocyte adhesion and platelet aggregation, contributing to plaque destabilization and thrombotic risk. GDF15, a stress-responsive cytokine, is associated with adverse cardiovascular outcomes, linking metabolic dysfunction to chronic inflammation. Likewise, inflammasome activation, particularly through NACHT, LRR, and PYD domains-containing protein 3 and absent in melanoma 2 pathways, triggers cytokine cascades that perpetuate vascular injury, while clonal hematopoiesis of indeterminate potential promotes myeloid-driven inflammation and atherosclerotic acceleration. The expanding role of these biomarkers underscores the complexity of inflammation in ASCVD and highlights their potential for refining cardiovascular risk assessment and elucidating novel mechanisms underlying plaque progression.

***Keywords:*** Cardiovascular disease; Chronic kidney disease; Atherosclerotic cardiovascular disease; Inflammatory biomarkers; Vascular inflammation; Plaque instability and thrombosis

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## 1. Introduction

### 1.1. The prevalence of cardiovascular disease

Globally, cardiovascular disease (CVD) remains the leading cause of mortality and morbidity, accounting for over 20.6 million deaths and approximately 393 million disability-adjusted life years annually.[1,2] While high-income countries have achieved a 34.9% decline in CVD mortality from 1990 to 2022, reflecting the impact of preventive measures and advancements in treatment, low- and middle-income countries are experiencing a rising burden. These alarming statistics underscore the urgent need for comprehensive strategies that combine population-level prevention, risk factor management, and equitable access to healthcare to mitigate the profound impact of CVD on global health systems.

### 1.2. Reduction in cardiovascular disease inflammation through risk factor control

Reducing the global burden of CVD requires prioritizing the control of modifiable risk factors to decrease disease prevalence, mortality, and healthcare costs. The American Heart Association's Life's Essential 8 framework provides a comprehensive roadmap for achieving and maintaining cardiovascular health (CVH) across diverse populations (Figure 1). The framework is as follows:

i. Diet: Adherence to dietary patterns such as the Dietary Approaches to Stop Hypertension or the Mediterranean diet lowers hypertension, atherogenic lipids, and ischemic risk by emphasizing whole foods, healthy fats, and low sodium
ii. Physical activity: Regular exercise boosts endurance, lowers blood pressure, enhances insulin sensitivity, and reduces inflammation, reducing the risk of myocardial infarction and heart failure
iii. Nicotine exposure: Avoiding smoking and secondhand smoke prevents endothelial dysfunction and atherogenesis, lowering the risk of peripheral arterial disease and coronary events
iv. Sleep health: Adequate sleep (7 – 9 h) supports CVH by regulating metabolism, reducing sympathetic overactivity, and mitigating hypertension and obesity
v. Body mass index (BMI): Maintaining a healthy BMI lowers the prevalence of metabolic syndrome and reduces the risk of coronary artery disease and non-ischemic cardiomyopathy
vi. Blood lipids: Controlling non-high-density lipoprotein (HDL) cholesterol slows atherosclerosis progression and stabilizes plaques, especially in high-risk individuals
vii. Blood glucose: Optimized glucose control prevents diabetes-related vascular complications, including retinopathy, nephropathy, myocardial infarction, and stroke
viii. Blood pressure: Managing systolic and diastolic blood pressure prevents strokes, heart failure, and renal damage, reducing the overall CVD burden.

Studies indicate that achieving high scores across these domains reduces CVD risk by up to 80% and could prevent an estimated 2 million CVD events annually in the United States (U.S.) alone.[3] A recent analysis highlighted the significant impact of adhering to the updated Life's Essential 8 framework in improving CVH and reducing mortality, demonstrating a linear association between CVH scores and reductions in all-cause and CVD-specific mortality. Among a cohort of nearly 20,000 U.S. adults, those achieving high CVH scores (≥75) experienced a 58% reduced risk of all-cause mortality and a 64% reduced risk of CVD-specific mortality compared to those with low scores.[4] Physical activity emerged as the most significant contributor, with improvements linked to a 17.8% reduction in CVD-specific mortality. Similarly, optimal control of blood pressure and glucose were critical, accounting for 12.5% and 10.3% of preventable deaths, respectively.

## 2. Inflammation in atherosclerotic cardiovascular disease (ASCVD)

### 2.1. The inflammatory mechanisms underlying ASCVD pathogenesis

Inflammation is now recognized as a key driver of ASCVD, redefining it from a passive lipid accumulation process to a dynamic interplay of immune activation and vascular injury. Biomarkers such as high-sensitivity C-reactive protein (hs-CRP) highlight the residual inflammatory risk, even in patients with optimal low-density lipoprotein (LDL) levels on statins.

### 2.2. Plaque formation

The formation of atherosclerotic plaques is initiated by endothelial injury (Figure 2). Endothelial dysfunction promotes the retention of apolipoprotein B-containing lipoproteins, such as LDL, within the subendothelial space. These lipoproteins undergo oxidative modification, triggering the expression of adhesion molecules, such as vascular cell adhesion molecule 1 and intercellular adhesion molecule 1, which facilitate the recruitment of circulating monocytes.[5,6] On transmigration into the intima, monocytes differentiate into macrophages and engulf oxidized LDL, forming foam cells. Concurrently, smooth muscle cells migrate from the media to the intima and synthesize extracellular matrix components, such as collagen, which stabilize the nascent plaque. However,

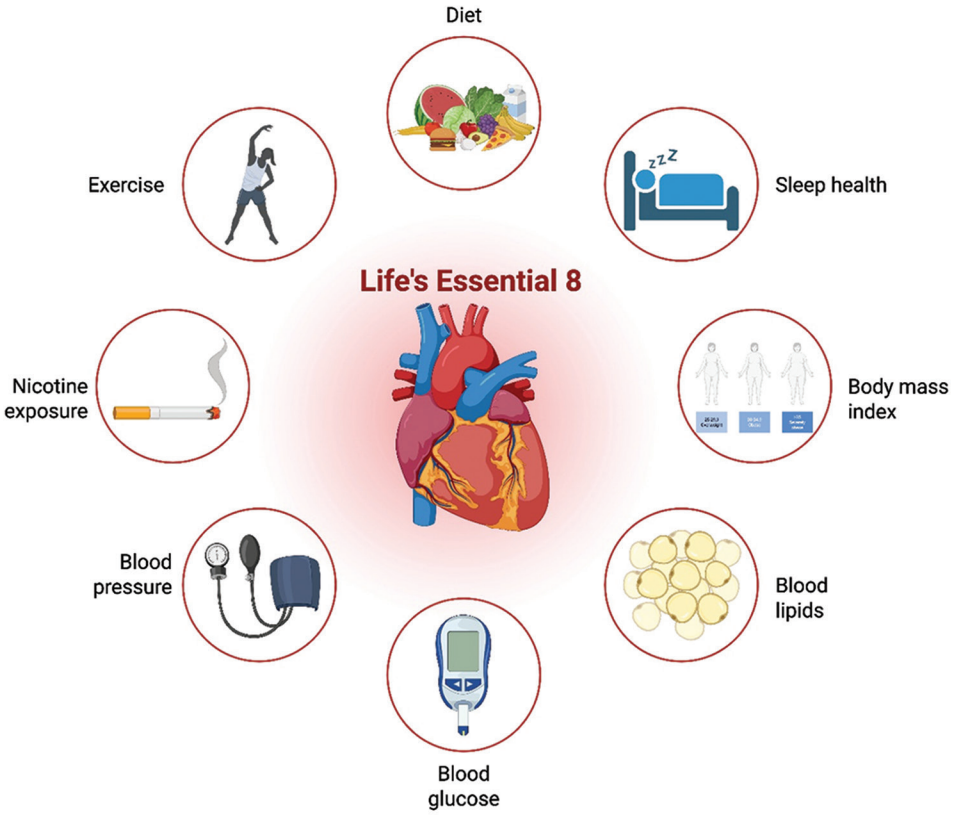


**Figure 1.** Life's Essential 8 for optimal cardiovascular health. These include a heart-healthy diet, engaging in regular physical activity, avoiding nicotine exposure, maintaining restorative sleep hygiene, achieving a healthy body mass index, optimizing blood pressure, controlling blood lipids, and managing blood glucose levels. Collectively, these factors form the foundation for reducing the burden of atherosclerotic cardiovascular disease and associated complications. Image was created using Biorender.

a chronic inflammatory environment leads to apoptotic cell death and necrosis, forming a lipid-rich necrotic core surrounded by calcified deposits.

### 2.3. Plaque rupture

Rupture occurs when the structural integrity of the fibrous cap is compromised, exposing the highly thrombogenic necrotic core to circulating blood. This event is predominantly driven by macrophage infiltration into the cap, where these cells release matrix metalloproteinases and other proteolytic enzymes that degrade collagen and weaken the extracellular matrix.[5,6] Simultaneously, the apoptosis of smooth muscle cells further reduces collagen synthesis, exacerbating cap thinning. Thin-cap fibroatheromas, characterized by fibrous caps <65 μm thick, large necrotic cores, and extensive macrophage infiltration, are particularly prone to rupture. The exposure to necrotic material activates platelets and the coagulation cascade, leading to thrombus formation that can occlude arterial blood flow, resulting in myocardial infarction.

### 2.4. The role of inflammatory markers

Inflammatory blood markers provide critical insights into ASCVD pathophysiology, reflecting systemic immune activation and its role in disease progression (Table 1). The interleukin (IL)-1 cytokine signaling, particularly through IL-1β, serves as a key upstream mediator of inflammation by activating nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase/protein kinase B pathways, leading to increased IL-6 transcription. This cascade amplifies systemic inflammation, with IL-6 stimulating the hepatic synthesis of hs-CRP, a widely studied biomarker of cardiovascular risk. Hs-CRP levels have been incorporated into risk stratification tools, such as the Reynolds Risk Score, and have been shown to improve the identification of individuals at heightened ASCVD risk, particularly those with low LDL levels but persistent low-grade inflammation.[5] Elevated IL-6 levels, a cytokine that contributes to atherogenesis by promoting endothelial activation and foam cell formation, have also been linked to a two-fold increase in cardiac events among individuals in the highest quartile of IL-6 levels.

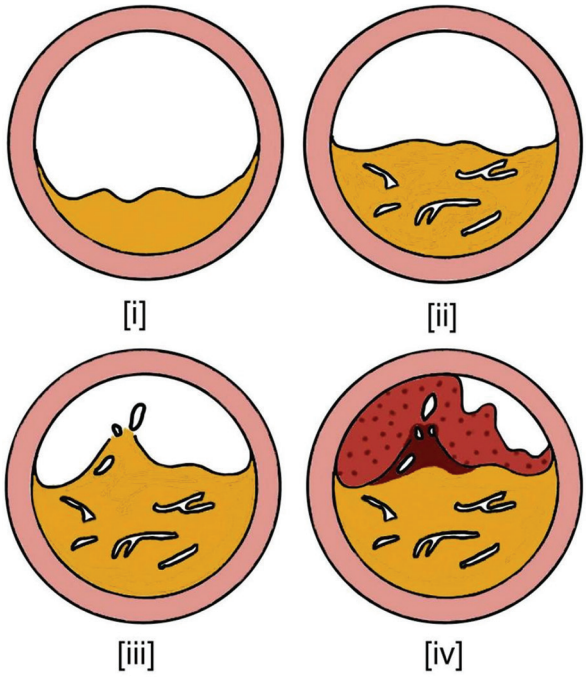


**Figure 2.** Four stages of atherosclerosis, from fatty deposition to plaque rupture: (i) Early fatty streak formation: Initial accumulation of lipids in the arterial wall. (ii) Plaque expansion: Increased lipid accumulation with inflammatory cell infiltration. (iii) Advanced plaque: Plaque enlargement with cholesterol deposits and immune cell involvement. (iv) Plaque rupture and thrombosis: Ulceration and rupture of the plaque, leading to potential clot formation and vessel occlusion. Image was created using Notability.

Additional biomarkers offer further insights into ASCVD pathophysiology. Myeloperoxidase (MPO) released by inflammatory cells catalyzes LDL and HDL oxidative modifications, impairing cholesterol efflux and contributing to plaque instability.[5] Lipoprotein-associated phospholipase A2 (Lp-PLA2), primarily associated with LDL particles, promotes oxidative stress and the progression of atherosclerotic plaques by releasing pro-inflammatory mediators. In large cohort studies, elevated plasma levels of Lp-PLA2 have been independently linked to coronary artery disease events.[5] Finally, trimethylamine-N-oxide, a gut microbiome-derived metabolite, exacerbates vascular inflammation by promoting cholesterol deposition and foam cell formation, correlating with adverse cardiovascular outcomes.

### 2.5. Emerging biomarkers in ASCVD

While established biomarkers, such as hs-CRP and IL-6, help assess systemic inflammation, emerging biomarkers provide deeper insight into disease mechanisms. These include cluster of differentiation (CD)47, serum and glucocorticoid-regulated kinase 1 (SGK1), P-selectin, and growth differentiation factor 15 (GDF15).

In immune regulation, CD47, widely expressed on vascular endothelial cells, macrophages, and platelets, plays a crucial role by interacting with signal regulatory protein-alpha (SIRPα) on phagocytes.[7] This interaction generates a "do not eat me" signal that suppresses macrophage-mediated clearance of apoptotic cells. In the context of ASCVD, increased CD47 expression within plaques promotes immune evasion, allowing damaged endothelial and lipid-laden foam cells to persist, fueling chronic inflammation and plaque progression.[7] Impaired efferocytosis leads to necrotic core expansion, further increasing the risk of plaque rupture and thrombosis. This dysfunction is mediated through the thrombospondin-1/CD47/SIRPα signaling, which limits apoptotic cell clearance and enhances IL-1β release through inflammasome activation, amplifying vascular immune responses.[7] Elevated CD47 levels correlate with plaque vulnerability, supporting its potential role in inflammatory risk stratification. Preclinical studies have demonstrated that CD47 blockade restores efferocytosis and reduces plaque burden, underscoring its emerging relevance as a prognostic biomarker and therapeutic target in ASCVD.

Besides, SGK1 is a serine/threonine kinase involved in ion transport, cellular survival, and inflammatory signaling. In ASCVD, it contributes to vascular remodeling by modulating endothelial function, promoting smooth muscle proliferation, and activating immune cells.[8] SGK1 enhances NF-κB signaling and NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome activation, leading to increased IL-6 and IL-1β production and subsequent leukocyte recruitment. It also disrupts endothelial nitric oxide synthase activity, promoting endothelial dysfunction and impaired vasodilation. In advanced plaques, SGK1-driven smooth muscle proliferation promotes neointimal thickening and arterial stiffening, exacerbating the hemodynamic burden of atherosclerosis. Inhibition of SGK1 in preclinical models has been shown to suppress NF-κB activity, reduce caspase-1 activation, and dampen downstream cytokine production, resulting in attenuation of cardiac inflammation and remodeling.[8] SGK1 expression is also elevated in thrombi from patients with acute myocardial infarction, suggesting potential utility in identifying high-risk inflammatory states. These findings highlight SGK1 as a mechanistic driver of vascular inflammation and a promising biomarker and therapeutic target in inflammation-mediated CVD.

Similarly, P-selectin, an adhesion molecule stored in the α-granules of platelets and Weibel–Palade bodies of endothelial cells, is rapidly translocated to the cell surface on activation, where it binds to P-selectin glycoprotein

**Table 1. Pro-inflammatory markers and their mechanisms in atherosclerotic inflammation**

| Pro-inflammatory marker | Mechanisms in atherosclerotic inflammation |
|---|---|
| Interleukin (IL)-1α | IL-1α functions as an alarmin released upon necrotic cell death. It is constitutively expressed in endothelial and epithelial cells. It binds to IL-1 receptor type 1 (IL-1R1) to activate NF-κB, MAPK, and JNK pathways, promoting early local inflammation, leukocyte recruitment, and induction of IL-1β. It is central to initiating the IL-1-driven inflammatory loop in ASCVD. |
| IL-1β | The binding of IL-1β to IL-1R1 activates signal pathways such as NF-κB, JNK, and p38 MAPK and induces expressions of genes such as IL-6, IL-8, MCP-1, COX-2, IL-1α, and IL-1β. |
| IL-6 | Multi-modal pathways are noted. IL-6 binds to membrane-bound IL-6 receptors present on hepatocytes, some leukocytes, and endothelial cells (classic signaling); to soluble forms of the IL-6 receptors, which allow signaling in most other cell types (trans-signaling); or through trans-presentation from dendritic cells to T cells (trans-presentation). All modes of signaling converge on the membrane signal-transducing receptor subunit glycoprotein 130 to activate the intracellular Janus kinase signal transducer and activator of transcription pathway. |
| Myeloperoxidase (MPO) | Released by inflammatory cells, MPO catalyzes oxidative modifications of LDL and HDL, impairing cholesterol efflux and destabilizing plaques. |
| High-sensitivity C-reactive protein (hs-CRP) | Synthesized in the liver in response to IL-6 activation, hs-CRP serves as a marker of systemic inflammation and cardiovascular risk. |
| Lipoprotein-associated phospholipase A2 (Lp-PLA2) | Lp-PLA2 is associated with LDL particles. It promotes oxidative stress and releases pro-inflammatory mediators, driving plaque progression. |
| Trimethylamine-N-oxide (TMAO) | Derived from the gut microbiome, TMAO enhances vascular inflammation by promoting cholesterol deposition and foam cell formation. |

Abbreviations: ASCVD: Atherosclerotic cardiovascular disease; COX-2: Cyclo-oxygenase-2; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; MCP-1: Monocyte chemoattractant protein-1; NF-κB: Nuclear factor-kappa B.

ligand-1 (PSGL-1) on monocytes and neutrophils.[9] This interaction facilitates leukocyte rolling, adhesion, and infiltration into the arterial intima, promoting local inflammation, foam cell formation, and plaque progression. Sustained P-selectin/PSGL-1 signaling activation amplifies cytokine release, fosters platelet–leukocyte aggregation, and contributes to both microvascular dysfunction and thrombotic instability. Genetic polymorphisms in the *SELP* gene have been associated with elevated soluble P-selectin levels and increased cardiovascular risk, suggesting a role in inflammatory risk stratification.[9] Furthermore, anti-P-selectin therapies, such as crizanlizumab and inclaclumab, have demonstrated early promise in reducing myocardial injury and dampening inflammatory thrombotic responses, underscoring the translational potential of this pathway in ASCVD.

Meanwhile, GDF15, a member of the transforming growth factor-beta superfamily, is a stress-responsive cytokine upregulated in response to ischemic injury, pressure overload, oxidative stress, and systemic inflammation. It integrates signals from cardiometabolic, inflammatory, and mechanical stress pathways, making it a broad cardiovascular risk marker. Persistently elevated GDF15 levels reflect maladaptive remodeling and are independently associated with adverse outcomes in coronary artery disease, heart failure, and atrial fibrillation – even after adjustment for natriuretic peptides and troponins.[10] Its prognostic value across large cohorts has positioned GDF15 as a promising tool for inflammatory risk stratification. In addition, the incorporation of GDF15 into multi-marker models improves the prediction of mortality and hospitalization, reinforcing its utility in identifying patients with high-risk, inflammation-driven CVD.

### 2.6. The role of adaptive immunity

The adaptive immune system plays a pivotal role in the pathogenesis of ASCVD, contributing to the progression and regulation of atherosclerotic plaques. The key components of this system include antigen-presenting cells, $CD4^+$ T-helper cells, and B lymphocytes, all of which orchestrate targeted immune responses within the arterial wall.

Antigen-presenting cells, such as dendritic cells and macrophages, process and present oxidized LDL and other atherogenic antigens through major histocompatibility complex molecules to naive T cells.[11,12] This interaction drives the differentiation of $CD4^+$ T cells into distinct subsets with varying impacts on atherogenesis. T helper

1 cells, the predominant subset in plaques, release pro-inflammatory cytokines, such as interferon-γ and tumor necrosis factor-alpha (TNF-α), which amplify vascular inflammation, recruit additional immune cells, and destabilize plaques by promoting matrix degradation and necrotic core expansion.

Meanwhile, B lymphocytes also play a pivotal role in the progression of ASCVD by amplifying inflammatory responses within atherosclerotic plaques. Adaptive B2 cells contribute to disease pathogenesis by producing IgG antibodies against atherogenic antigens, which form immune complexes and activate complement pathways, intensifying local vascular inflammation.[11,12] These cells secrete pro-inflammatory cytokines, such as IL-6 and TNF-α, further exacerbating endothelial dysfunction and promoting plaque instability. By interacting with other immune cells and perpetuating inflammatory cascades, B lymphocytes contribute significantly to the chronic immune activation that drives the progression and destabilization of atherosclerotic plaques in ASCVD.

The clonal expansion of T- and B-cells specific to atherogenic antigens sustains the immune activation within plaques, perpetuating a cycle of inflammation and vascular injury. The accumulation of immune complexes, activation of complement pathways, and engagement of cytotoxic $CD8^+$ T cells further contribute to plaque destabilization, emphasizing the multifaceted nature of active immunity in ASCVD.

## 3. The role of inflammasomes in ASCVD

### 3.1. Introduction to inflammasomes in ASCVD

Inflammasomes are multiprotein complexes within the cytoplasm that act as pivotal regulators of the inflammatory response. They link cellular stress and immune activation in the development and progression of ASCVD. The NLRP3 and absent in melanoma 2 (AIM2) inflammasomes are the most extensively studied inflammasomes implicated in ASCVD (Figure 3). Together, these inflammasomes destabilize atherosclerotic plaques, making them more prone to rupture and thrombosis.

### 3.2. Mechanisms of NLRP3 and AIM2 inflammasomes

The NLRP3 inflammasome is a central driver of inflammation in atherosclerosis and is activated through a well-characterized two-step process. The priming phase is initiated by pattern recognition receptors, such as toll-like receptors, which activate NF-κB and upregulate the transcription of NLRP3 and its downstream effectors, pro-IL-1β and pro-IL-18.[13,14] The subsequent activation phase is triggered by stimuli characteristic of the atherogenic environment, including extracellular cholesterol crystals, oxidized LDL, potassium efflux, lysosomal destabilization, and mitochondrial-derived reactive oxygen species. These signals converge to promote NLRP3 oligomerization and its interaction with the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase-1, forming the active inflammasome complex. Once assembled, this complex cleaves pro-caspase-1 into its active form, which subsequently processes pro-IL-1β and pro-IL-18 into their mature, secreted forms. These cytokines propagate vascular inflammation by inducing endothelial cell activation, upregulating adhesion molecules, and recruiting monocytes to the arterial intima. In vascular smooth muscle cells, inflammasome activity promotes apoptosis and extracellular matrix degradation, while in macrophages, inflammasome-driven IL-1β release enhances foam cell formation and necrotic core expansion. Together, these effects compromise fibrous cap integrity, reduce plaque stability, and increase susceptibility to rupture and thrombosis.

The AIM2 inflammasome operates through a distinct DNA-sensing mechanism, detecting cytoplasmic double-stranded DNA (dsDNA) derived from mitochondrial damage, necrotic cells, or neutrophil extracellular traps.[13,14] On binding dsDNA, AIM2 undergoes conformational changes that drive its oligomerization and subsequent recruitment of ASC and pro-caspase-1, leading to caspase-1 activation and downstream cytokine maturation. In macrophages and endothelial cells, AIM2 activation has been shown to induce pyroptotic cell death through gasdermin D pore formation, disrupting membrane integrity and releasing intracellular damage-associated molecular patterns (DAMPs), such as DNA fragments, ATP, and oxidized lipids.[13,14] These DAMPs perpetuate local inflammation and act as secondary triggers for additional inflammasome activation, creating a self-reinforcing inflammatory loop within the plaque. AIM2 has also been implicated in promoting plaque necrosis, expanding the necrotic core, and impairing resolution pathways such as efferocytosis – further destabilizing the plaque microenvironment. The combined activity of NLRP3 and AIM2 inflammasomes across multiple vascular cell types highlights their role as central orchestrators of atherosclerotic progression and potential therapeutic targets for inflammation-driven CVD.

### 3.3. The IL-1 cytokine signaling: Upstream initiator of inflammation

Before amplifying downstream mediators such as IL-6, the IL-1 family – particularly IL-1α and IL-1β – initiates

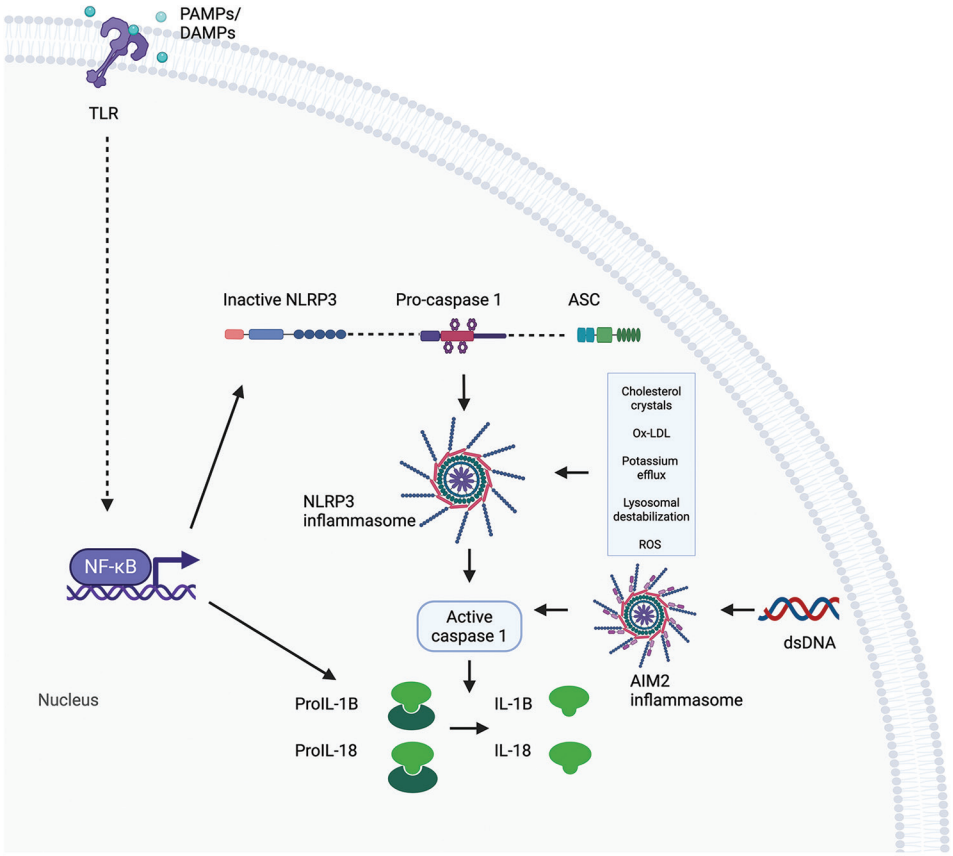


**Figure 3.** Inflammasome pathways in atherosclerosis. The NACHT, LRR, and PYD domains-containing protein 3 inflammasome activation involves toll-like receptor-mediated priming via nuclear factor-kappa B and triggering by stimuli such as cholesterol crystals, oxidized low-density lipoprotein, and reactive oxygen species. This leads to caspase-1 activation and interleukin (IL)-1β/IL-18 maturation. The absent in melanoma 2 inflammasome, activated by cytoplasmic double-stranded DNA, also promotes IL-1β/IL-18 release and pyroptosis. Together, these pathways drive chronic inflammation. Image was created using Biorender. Abbreviations: ASC: Apoptosis-associated speck-like protein containing a CARD; DAMPs: Damage-associated molecular patterns; PAMPs: Pathogen-associated molecular patterns.

vascular inflammation. IL-1α is constitutively expressed in endothelial and epithelial cells and is rapidly released in a biologically active form on cell necrosis, functioning as an alarmin that alerts surrounding immune cells to tissue injury.[15] In contrast, IL-1β is synthesized by activated myeloid cells and requires cleavage by the NLRP3 inflammasome to become active. Both forms engage the IL-1 receptor type 1, triggering NF-κB and MAPK signaling cascades that upregulate adhesion molecules, cytokines, and chemokines critical to monocyte recruitment and endothelial dysfunction. Experimental models demonstrate that IL-1α release precedes IL-1β activation, initiating an IL-1-driven loop in which dying vascular cells stimulate infiltrating macrophages to produce IL-1β, further amplifying local and systemic inflammation.[15] This interplay is crucial in atherosclerosis, where IL-1 signaling promotes foam cell formation, plaque destabilization, and thrombotic risk. Importantly, IL-1 directly induces hepatic IL-6 production, establishing a mechanistic bridge between early innate immune activation and downstream cytokine-mediated risk.

The upstream positioning of IL-1α and IL-1β in the inflammatory cascade has translational implications. Elevated IL-1 levels have been detected in patients with acute coronary syndromes, myocarditis, and inflammatory vascular conditions, supporting its role as a clinical biomarker of vascular inflammation. Moreover, targeted inhibition of IL-1 signaling with agents, such as anakinra, rilonacept, and canakinumab, has shown benefits across a range of cardiovascular and systemic inflammatory diseases. These findings underscore the diagnostic and therapeutic potential of IL-1 modulation in inflammation-driven cardiovascular pathology. With the upstream role of IL-1 established, attention now turns to IL-6, whose broad influence across cardiovascular, renal, and metabolic systems underscores its relevance as both a biomarker and a therapeutic target.

## 4. IL-6 inhibition in cardiac disease and chronic kidney disease (CKD): A multidimensional perspective

### 4.1. Introduction to IL-6 signaling

IL-6 is pivotal in immune regulation and inflammatory responses, acting through complex signaling pathways. It is secreted by various cell types, including macrophages, fibroblasts, and endothelial cells, often in response to upstream stimuli, such as IL-1 or tissue injury. IL-6 signals through three distinct mechanisms: (i) classic signaling, in which IL-6 binds to membrane-bound IL-6 receptors and activates glycoprotein 130 (gp130); (ii) trans-signaling, which uses soluble IL-6 receptors to broaden its effect to cells lacking IL-6 receptors; and (iii) trans-presentation, a specialized process involving dendritic cells and T cells.[16] All signaling modes converge on gp130 and activate the Janus kinase signal transducer and activator of transcription (JAK-STAT) pathway, driving gene expression changes that promote inflammation, endothelial dysfunction, and disease progression. Beyond its mechanistic roles, IL-6 has emerged as a potent clinical biomarker and therapeutic target in inflammation-driven CVD. Elevated IL-6 levels are independently associated with adverse outcomes in coronary artery disease, heart failure, and CKD, even after adjusting for traditional risk markers. Genetic evidence supports a causal relationship between IL-6 signaling and ASCVD, while *post hoc* analyses from the CANTOS trial demonstrated that a reduction in IL-6 levels was closely tied to improved cardiovascular outcomes.[16] These insights have led to the development of IL-6-targeted therapies, such as ziltivekimab, which is currently being tested in the ZEUS trial (ClinicalTrials.gov ID: NCT05021835) to reduce cardiovascular events in patients with CKD and elevated inflammatory risk.

### 4.2. The role of IL-6 in acute coronary ischemia

Acute coronary ischemia, characterized by myocardial hypoxia and plaque rupture, is significantly affected by elevated IL-6 levels, which amplify systemic and local inflammation. In phase II trials, including the ASSAIL-MI and SOLID-TIMI 52[17] studies, a single administration of the IL-6 receptor antagonist tocilizumab in patients with ST-segment elevation myocardial infarction demonstrated reductions in microvascular obstruction and inflammatory markers, such as CRP. These improvements were evident during hospitalization and were particularly notable when treatment was initiated after a delay of more than 3 h from symptom onset. Mechanistically, IL-6 inhibition mitigates the acute-phase response by decreasing monocyte chemoattraction and neutrophil activation, thereby limiting inflammatory damage to myocardial tissue.

### 4.3. Integrative inflammatory networks in ASCVD

While each inflammatory pathway contributes uniquely to atherogenesis, their interactions form a tightly interconnected network that amplifies vascular injury and plaque progression. IL-1 signaling, often triggered by necrotic cell death or inflammasome activation, initiates a cascade that upregulates IL-6, bridging local tissue inflammation with systemic responses, such as hepatic hs-CRP production. IL-6, in turn, reinforces monocyte activation and endothelial dysfunction, enhancing the expression of adhesion molecules and facilitating leukocyte infiltration. Concurrently, SGK1 intensifies this pro-inflammatory signaling environment by promoting NF-κB activity and NLRP3 inflammasome assembly, further increasing IL-1β and IL-6 production. CD47 exacerbates this cycle by impairing efferocytosis, allowing apoptotic cells and inflammatory debris to accumulate within the plaque, thereby sustaining immune cell activation. In addition, MPO and Lp-PLA2 perpetuate oxidative stress, leading to destabilized plaques and amplified cytokine release. These interwoven pathways collectively fuel a feed-forward loop of inflammation, oxidative injury, and immune dysregulation – underpinning the pathophysiology of plaque instability and thrombotic events in ASCVD.

The downstream consequences of these overlapping inflammatory circuits become especially pronounced in chronic cardiovascular and renal conditions, where IL-6 drives maladaptive tissue remodeling, systemic immune activation, and end-organ dysfunction – hallmarks of both heart failure and CKD.

### 4.4. The role of IL-6 in heart failure

IL-6 plays a central role in heart failure, promoting adverse cardiac remodeling through inflammatory pathways. Elevated IL-6 levels have been consistently associated with higher rates of hospitalization, cardiovascular mortality, and worsening cardiac function. Findings from the BIOSTAT-CHF study revealed that IL-6 correlated with markers of disease severity, including elevated N-terminal pro-B-type natriuretic peptide and cardiac fibrosis.[16,17] Moreover, IL-6 stimulates hepcidin production, contributing to anemia – a common exacerbating factor in heart failure.[16,17] Clinical evidence from the CANTOS trial demonstrated that patients with the highest reductions in IL-6 following anti-inflammatory therapy exhibited the most significant declines in heart failure hospitalizations.

### 4.5. The role of IL-6 in CKD and hemodialysis

Patients with CKD and those undergoing hemodialysis experience elevated IL-6 levels due to persistent oxidative

stress, tissue hypoxia, and retention of uremic toxins.[16,17] IL-6 predicts vascular events and mortality more accurately than traditional lipid measures in prognostic accuracy. Elevated IL-6 levels contribute to systemic inflammation, exacerbating endothelial dysfunction and promoting atherosclerosis. In hemodialysis patients, IL-6 also affects anemia by modulating hepcidin levels, reducing iron bioavailability, and impairing erythropoiesis.[16,17] Data from the CANTOS trial and related studies suggest that targeting IL-6 pathways reduces inflammatory marker expression and may improve cardiovascular outcomes in CKD patients.

## 5. Clonal hematopoiesis as a risk factor for ASCVD

### 5.1. Clonal hematopoiesis of indeterminate potential (CHIP) and atherosclerosis

CHIP is characterized by somatic mutations in hematopoietic stem and progenitor cells (HSPCs), increasingly recognized as a key contributor to ASCVD. Frequently observed mutations – most commonly in *TET2*, *DNMT3A*, *ASXL1*, and *JAK2* – promote myeloid-biased differentiation and endow HSPCs with enhanced self-renewal and inflammatory potential. These mutations are not merely passenger events. They induce functional changes in immune cells that accelerate atherosclerosis. In murine models, the transplantation of *Tet2*-deficient bone marrow promotes plaque growth, enhanced macrophage accumulation, and increased IL-1β secretion, mainly through NLRP3 inflammasome activation, even when present in only 10% of donor marrow.[18-20] This indicates that minor clonal populations can exert outsized inflammatory effects. Similarly, *Jak2* V617F mutation increases monocyte recruitment, macrophage proliferation, and necrotic core formation in plaques, driven by dual activation of AIM2 and NLRP3 inflammasomes. CHIP-mutant macrophages exhibit a hyperinflammatory phenotype that compromises efferocytosis, destabilizes plaques, and enhances leukocyte recruitment. These effects are not limited to atherosclerosis alone but extend to impaired cardiac repair and increased fibrosis in heart failure models. Epidemiologically, CHIP carriers – especially those with high variant allele fractions – face a twofold increased risk of ASCVD, comparable to traditional risk factors and independent of lipid levels or smoking history.[18-20] Emerging evidence also links CHIP to epigenetic aging, suggesting an additional biomarker framework for risk stratification.

### 5.2. Gene variants associated with clonal hematopoiesis of indeterminate potential

The pathogenicity of CHIP is highly dependent on the specific mutated gene, each of which affects inflammatory pathways through distinct mechanisms. *TET2* mutations lead to the loss of its demethylase function, increasing histone acetylation at inflammatory gene promoters, such as those in *IL1B* and *NLRP3*. *Tet2*-deficient macrophages display enhanced inflammasome priming and IL-1β production in response to oxidized LDL and other danger signals.[18-20] Treatment with NLRP3 inhibitors in murine *Tet2*-CHIP models significantly attenuates plaque burden, underscoring the therapeutic potential of targeting upstream inflammatory pathways. *DNMT3A* mutations, while impairing epigenetic regulation, alter gene expression through distinct methylation patterns. Although they similarly skew hematopoiesis and promote a pro-inflammatory macrophage phenotype, *DNMT3A*-mutant cells show increased IL-6 and chemokine secretion with relatively less IL-1β production, suggesting partial divergence from Tet methylcytosine dioxygenase 2 (Tet2)-mediated pathways.[18-20] *JAK2* V617F mutation, a gain-of-function mutation, enhances cytokine signaling through STAT pathways and drives NLRP3 and AIM2 inflammasome activation. *Jak2*-mutant macrophages demonstrate metabolic reprogramming – elevated glycolysis and mitochondrial reactive oxygen species production – that promotes pyroptosis and plaque instability.[18-20] In contrast to TET2, AIM2 appears to play a more dominant role in JAK2-associated atherogenesis. DNA damage response gene mutations, such as *TP53* and *PPM1D*, also contribute by expanding inflammatory myeloid populations, though without consistent inflammasome activation, suggesting inflammasome-independent contributions to plaque growth.[18-20] Collectively, these mutation-specific pathways point to a nuanced immunoepigenetic landscape and support the development of tailored anti-inflammatory therapies targeting CHIP-associated atherosclerosis.

## 6. Conclusion

Inflammation drives ASCVD through complex immune pathways, including cytokine signalings, inflammasome activation, and clonal hematopoiesis. Biomarkers such as hs-CRP, IL-1, IL-6, and MPO, as well as emerging targets such as CD47 and SGK1, provide critical insights into disease progression and risk stratification. Understanding these mechanisms refines our ability to predict cardiovascular events and uncover novel pathogenic pathways. Future research should focus on integrating these biomarkers to enhance diagnostic precision and deepen our understanding of inflammatory drivers in CVD. As the first part of a two-part review, this article outlines the immunopathogenic mechanisms and biomarker landscape of inflammation in CVD, while Part II will focus on therapeutic strategies targeting inflammatory pathways.

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## Ethics approval and consent to participate

Not applicable.

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## Further disclosure

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REVIEW ARTICLE

# Inflammation and cardiovascular disease – Part II: Anti-inflammatory therapy in cardiovascular disease

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## Abstract

Inflammation plays a central role in the pathogenesis of atherosclerotic cardiovascular diseases (ASCVDs), contributing to plaque progression, instability, and thrombosis. Chronic systemic inflammation exacerbates endothelial dysfunction, promotes oxidative stress, and accelerates atherogenesis, necessitating targeted interventions. This review explores established and emerging strategies for modulating inflammation to improve cardiovascular outcomes. Statin therapy remains foundational, with trials, such as JUPITER, demonstrating significant reductions in cardiovascular events through high-sensitivity C-reactive protein modulation, independent of low-density lipoprotein lowering. Non-statin lipid-lowering therapies, including proprotein convertase subtilisin/kexin type 9 inhibitors, ezetimibe, and bempedoic acid, have shown additional anti-inflammatory benefits and further reduce inflammation-driven cardiovascular risk. In addition, triglyceride-lowering agents targeting apolipoprotein C-III and angiopoietin-like protein pathways offer promising avenues for reducing metabolic inflammation and residual ASCVD risk. Anti-inflammatory pharmacotherapy has gained traction, with trials such as canakinumab anti-inflammatory thrombosis outcomes study, colchicine cardiovascular outcomes trial, and low-dose colchicine underscoring the efficacy of canakinumab and colchicine in reducing cardiovascular events. Emerging interleukin (IL) pathways (e.g., IL-17, IL-33, and IL-36) and novel therapeutic targets (e.g., cluster of differentiation 47 inhibitors, serum/glucocorticoid-regulated kinase 1 modulation, and P-selectin blockade) present future opportunities for precision cardiovascular medicine. However, residual inflammatory risk persists despite optimal lipid control, highlighting the need for a multimodal approach integrating lipid-lowering, anti-inflammatory, and targeted immunomodulatory therapies. The expanding role of inflammation in ASCVD suggests a paradigm shift toward inflammation-guided treatment strategies. Further research is warranted to refine patient selection, personalize therapy, and optimize long-term outcomes for inflammation-driven cardiovascular disease.

***Keywords:*** Cardiovascular inflammation; Cardiovascular disease; Chronic kidney disease; Inflammation-targeted therapies; Lipid-lowering therapy; PCSK9 inhibitors and inflammation reduction; ApoC-III and triglyceride-lowering therapies

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## 1. Introduction: Managing inflammation as a key factor in atherosclerotic cardiovascular disease (ASCVD)

ASCVD is a leading global cause of mortality, responsible for over 17 million deaths annually.[1] Traditional risk factors, such as hyperlipidemia, hypertension, diabetes, and obesity, are well established, but increasing evidence highlights the pivotal role of systemic inflammation in disease progression and plaque instability.

The references cited in this review were identified through a structured literature search of PubMed and ClinicalTrials.gov from January 2000 to March 2025 using keywords such as "inflammation," "atherosclerosis," "cardiovascular disease," "interleukin," "ApoC-III," and "anti-inflammatory therapy." Additional studies were identified by manually reviewing the reference lists of relevant articles. Only peer-reviewed studies published in English were included, with priority given to randomized clinical trials, large cohort studies, and landmark translational investigations.

## 2. Evidence with statin therapy, low-density lipoprotein (LDL) reduction, and cardiovascular disease (CVD) prevention

Pravastatin has been studied extensively in the West of Scotland Coronary Prevention Study (WOSCOPS) and the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trials.[2] WOSCOPS showed a 26% reduction in LDL levels and a 28% reduction in cardiac mortality with pravastatin. The PROSPER trial showed a 34% reduction in LDL levels and a 24% reduction in cardiac outcomes in elderly patients. The Heart Protection Study evaluated the benefits of simvastatin in 20,000 patients with no prior coronary events and found an 18% reduction in cardiac mortality and a 26% reduction in coronary events in the simvastatin 40 mg daily group compared to the placebo group.[2]

Building upon prior evidence, the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin trial further evaluated statins in primary prevention, specifically in individuals with LDL levels <130 mg/dL but elevated high-sensitivity C-reactive protein levels (hs-CRP; ≥2.0 mg/L).[3] The trial enrolled 17,802 participants, who were randomized to receive either rosuvastatin 20 mg daily or placebo. Rosuvastatin therapy resulted in a 50% reduction in LDL levels, a 37% reduction in hs-CRP levels, and a 44% reduction in major cardiovascular events. The trial was terminated early due to significant cardiovascular risk reduction.

## 3. Beyond statins for LDL reduction

While statins remain the primary therapy for dyslipidemia, gene therapy offers emerging alternatives. Approaches include gene addition, inactivation, and editing of target hepatocytes to restore cholesterol metabolism. In pre-clinical studies, adeno-associated virus-mediated gene therapy has demonstrated a 98% reduction in cholesterol levels, showing promise for the treatment of familial hypercholesterolemia (Figure 1).[4]

Beyond statins, novel agents, such as ezetimibe, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, and bempedoic acid, have emerged to effectively treat dyslipidemia (Figure 2). Ezetimibe inhibits Niemann–Pick C1-like protein 1, a transporter of food cholesterol from the intestinal lumen into enterocytes, reducing LDL levels by 15 – 20%.[5] In the Improved Reduction of Outcomes: Vytorin Efficacy International Trial, adding ezetimibe to simvastatin resulted in a 24% reduction in LDL levels and a 2% decrease in cardiovascular events.[6] PCSK9 inhibitors (e.g., alirocumab and evolocumab) enhance LDL receptor recycling by preventing LDL receptor degradation and have resulted in >60% reduction in LDL levels in the Long-term Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy (ODYSSEY)[7] and the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trials.[8] Bempedoic acid, an ATP citrate lyase inhibitor, showed a 21% reduction in LDL levels in the Cholesterol Lowering through Bempedoic Acid (ECT1002), an ACL-Inhibiting Regimen (CLEAR) Outcomes trial, particularly benefiting statin-intolerant patients.[9] A subgroup analysis of patients with baseline hs-CRP levels >2 mg/L in the bempedoic acid group showed a median decrease of 1.66 mg/L in hs-CRP levels at the 12-week follow-up.[10]

## 4. Reduction of triglycerides and its impact on CVD

Genetic and pharmacological approaches targeting apolipoprotein C-III (ApoC-III), angiopoietin-like protein 3 (ANGPTL3), ANGPTL4, and related pathways show promise for reducing triglycerides. ApoC-III inhibition, initially observed in Amish populations with loss-of-function mutations, correlates with a 39% reduction in triglyceride levels and a 40% decrease in ASCVD risk.[11] ApoC-III also upregulates inflammatory cytokines (e.g., interleukin-6 [IL-6] and tumor necrosis factor-alpha [TNF-α]), contributing to endothelial dysfunction; therefore, inhibiting ApoC-III also reduces systemic inflammation, potentially preventing CVD progression.[12]

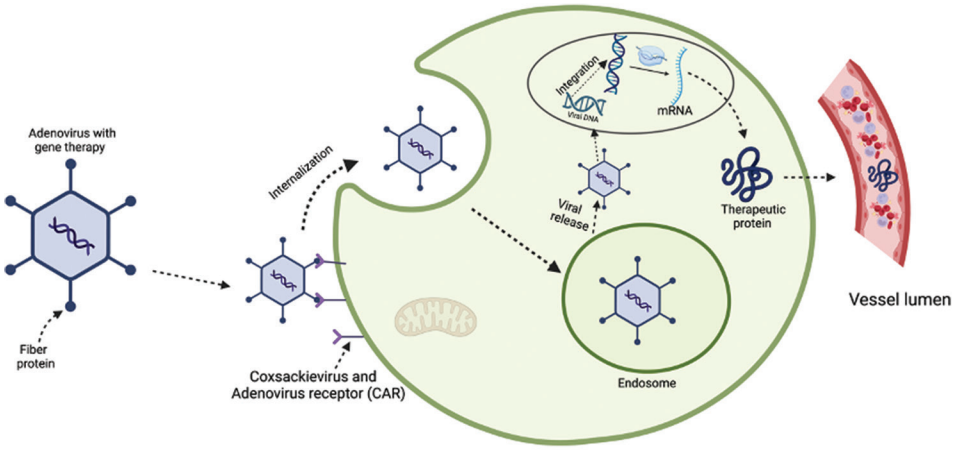


**Figure 1.** Adenovirus vector application in gene therapy. This method involves using a modified adenovirus to deliver a therapeutic gene into target cells. The virus facilitates cell entry and enables the integration of the targeted gene into the host genome. This leads to the desired production of therapeutic protein using the host cellular system at the molecular level. Image created by the authors with BioRender.com. Vipanpreet chahil (2025) https://app.biorender.com/illustrations/679fedb6b4efdea982d4ae03.

ApoC-III-mediated lipid dysregulation is a key driver of metabolic syndrome, a condition characterized by hypertriglyceridemia, low high-density lipoprotein (HDL) level, hypertension, and insulin resistance, affecting 35% of adults and half of those over 60 in the United States.[13] Metabolic syndrome significantly elevates CVD risk and contributes to nonalcoholic fatty liver disease (NAFLD), the leading cause of chronic liver disease, which impacts 30% of the global population. NAFLD is closely linked to obesity, insulin resistance, obstructive sleep apnea, and genetic predisposition.[14]

Hypertriglyceridemia management requires a multidisciplinary approach, emphasizing lifestyle interventions (e.g., diet control, exercise, and weight loss) and lipid-lowering agents. The class I recommendation advises identifying patients aged ≥20 years with hypertriglyceridemia and encouraging lifestyle changes.[15] Patients aged 40 – 75 years with moderate-to-severe hypertriglyceridemia (≥175 mg/dL) and ASCVD risk ≥7.5% should be reassessed after lifestyle modification. If triglyceride levels remain elevated, statin therapy is recommended. For high-risk patients with persistent triglyceride levels >200 mg/dL despite optimal treatment, the 2019 European Society of Cardiology and European Atherosclerosis Society guidelines support fibrates, PCSK9 inhibitors, and omega-3 fatty acids as add-on therapy.

## 5. Evidence of HDL modification and its impact on CVD

The primary functions of HDL include facilitating cholesterol efflux to the liver, delivering cholesterol to steroidogenic tissues, and mediating lipid exchange with apolipoprotein B (ApoB)-containing particles.[16] Beyond its lipid-modulating effects, HDL actively regulates inflammation, a key driver of atherosclerosis. HDL is typically anti-inflammatory but can exhibit transient pro-inflammatory properties, as observed in animal and human studies where its inflammatory activity peaks 3-day post-inflammation.[17] Under normal conditions, HDL suppresses vascular adhesion molecules and mitigates endothelial dysfunction, reinforcing its protective effects against atherogenesis.[18]

Examples of HDL-modifying agents are niacin, statins, cholesteryl ester transfer protein (CETP) inhibitors, and fibrates. Niacin effectively raises HDL levels but is limited by side effects, such as flushing. It reduces the levels of triglycerides, LDL, and total cholesterol while increasing HDL levels through inhibition of hepatocyte microsomal diacylglycerol acyltransferase-2 and selective inhibition of apolipoprotein A1 (ApoA1) uptake.[18] Fibrates are another option commonly used with statins, as they decrease triglyceride levels through hepatic synthesis of ApoA1 and ApoA2. However, targeting triglyceride levels reduction and increasing HDL levels has not demonstrated significant cardiovascular risk reduction.[18]

Cholesteryl esters are transferred by CETP from HDL to larger lipoproteins, lowering HDL levels.[18] Despite initial interest, CETP inhibitors have not demonstrated ASCVD risk reduction in major trials due to safety concerns or a lack of LDL-lowering effects. The Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification trial showed that anacetrapib increased HDL levels by 104% but did not reduce cardiovascular mortality or the incidence of major cardiac events.[18]

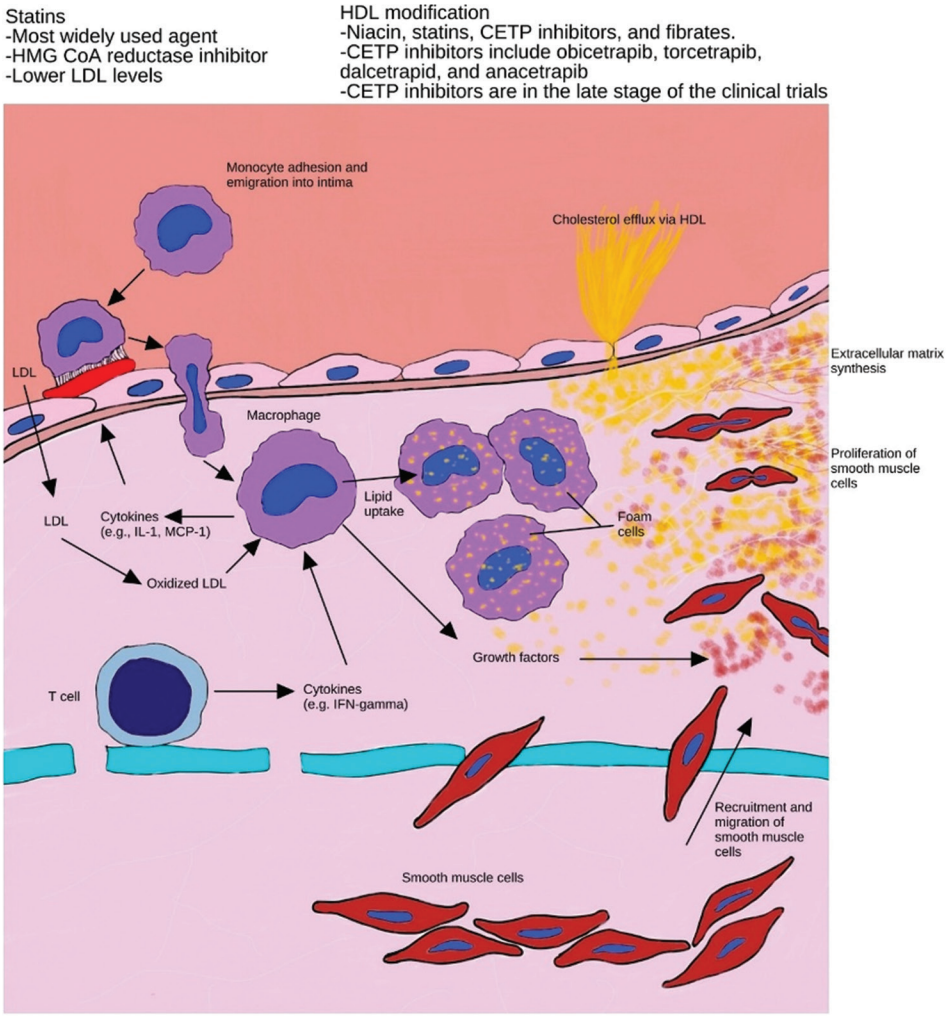


**Figure 2.** Pathophysiology and pharmacological interventions in atherosclerosis. This detailed diagram illustrates the key mechanisms in the development of atherosclerosis, highlighting the role of lipid accumulation, immune cell activation, and smooth muscle proliferation in plaque formation. It also presents various therapeutic strategies, including statins, HDL modification, gene therapy, bempedoic acid, eicosapentaenoic acid (EPA), and newer medications, such as ezetimibe and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Image created by the authors with notability.
Abbreviations: CETP: Cholesteryl ester transfer protein; HDL: High-density lipoprotein; HMG-CoA reductase: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IFN-gamma: Interferon-gamma; IL: Interleukin; LDL: Low-density lipoprotein; MCP-1: Monocyte chemoattractant protein 1; NF-κB: Nuclear factor kappa B; NPC1L1: Niemann–Pick C1-like protein 1; TNF-alpha: Tumor necrosis factor-alpha.

Obicetrapib, a newer CETP inhibitor, has shown improved safety and efficacy compared to previous agents. It significantly lowers the levels of LDL, non-HDL, ApoB, and lipoprotein (Lp[a]) while increasing the levels of pre-β HDL, mature HDL particles, and ApoA1. In phase 2 trials, the 10 mg obicetrapib group showed a 45% median reduction in LDL levels, a 34% reduction in ApoB levels, and a 33% decrease in Lp(a) levels.[19] Obicetrapib's clinical

program includes pivotal trials, such as the BROADWAY trial, which showed a 33% reduction in LDL levels and a 21% reduction in major adverse cardiovascular events (MACE) after 1 year.[20] The BROOKLYN trial demonstrated a 41.5% reduction in LDL levels in heterozygous familial hypercholesterolemia patients at 1 year.[20]

## 6. Eicosapentaenoic acid (EPA) and its anti-inflammatory benefits

EPA is crucial to cardiovascular health due to its anti-inflammatory and cardioprotective properties. It integrates into cell membranes, replacing arachidonic acid, a precursor to pro-inflammatory eicosanoids, such as prostaglandins and leukotrienes.[21] This shift reduces the production of inflammatory molecules and promotes the synthesis of anti-inflammatory mediators, such as resolvins, which are essential for resolving chronic inflammation. In addition, EPA downregulates key inflammatory cytokines, including TNF-α, IL-1, and IL-6, by inhibiting the nuclear factor kappa B (NF-κB) pathway, a major regulator of inflammation.

The Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial showed that high-dose icosapent ethyl led to a 25% reduction in MACE, including a 20% decrease in cardiovascular mortality and a 31% reduction in myocardial infarction incidence.[21] The trial also showed a significant reduction in inflammatory marker levels, with hs-CRP levels decreasing by 19% and IL-6 levels by 10%.

## 7. Clinical trials using anti-inflammatory agents in CVD prevention (CURT > low-dose colchicine [LODOCO], colchicine cardiovascular outcomes trial [COLCOT], canakinumab anti-inflammatory thrombosis outcomes study [CANTOS], stabilization of plaques using darapladib-thrombolysis in myocardial infarction [SOLID TIMI], and losmapimod in myocardial infarction 60 [LATITUDE TIMI])

Key clinical trials, including the Cardiovascular Inflammation Reduction Trial (CIRT), the LODOCO, the COLCOT, the CANTOS, the SOLID-TIMI 52, and the LATITUDE-TIMI 60 trials, have explored various inflammatory pathways in atherosclerosis and their impact on cardiovascular outcomes (Table 1).

The CIRT, a randomized placebo-controlled trial, examined low-dose methotrexate (15 – 20 mg weekly) in patients with prior myocardial infarction or multivessel coronary artery disease with metabolic syndrome or diabetes.[22] Despite its established role in autoimmune disease, methotrexate failed to reduce IL-1β, IL-6, or CRP levels and had no significant impact on cardiovascular outcomes. The primary endpoint occurred in an incidence rate of 4.13 versus 4.31/100 person-years in the methotrexate and placebo groups, respectively (hazard ratio [HR]: 0.96; 95% confidence interval [CI]: 0.79 – 1.16). These findings suggest that non-specific immunosuppression may not be effective in atherosclerosis.

In contrast, in post-myocardial infarction patients, the LODOCO trial demonstrated a 24% reduction in MACE with colchicine 0.5 mg daily treatment (HR: 0.76; 95% CI: 0.61 – 0.95).[23] Building on this, the COLCOT assessed colchicine's efficacy when initiated within 30-day post-myocardial infarction, showing a 23% reduction in cardiovascular events (HR: 0.77; 95% CI: 0.61 – 0.96), particularly in stroke and urgent revascularization, reinforcing its role in post-myocardial infarction management.[23]

The CANTOS trial explored anti-inflammatory therapy using canakinumab, a monoclonal antibody against IL-1β. In patients with elevated hs-CRP levels following myocardial infarction, treatment with canakinumab at 150 mg every 3 months reduced cardiovascular events by 15% (HR: 0.85; 95% CI: 0.74 – 0.98; $p$=0.021) and cardiovascular mortality by 21% (HR: 0.79; 95% CI: 0.67 – 0.93; $p$=0.005).[24] However, it did not reduce all-cause mortality (HR: 0.94; 95% CI: 0.83 – 1.06) and was associated with an increased risk of fatal infections, raising concerns about the safety of broad immune suppression.

The ZEUS trial is a randomized, double-blind cardiovascular outcomes study evaluating ziltivekimab, a monoclonal antibody that selectively inhibits IL-6, in patients with chronic kidney disease and ASCVD with elevated hsCRP levels ≥2 mg/L.[25] Given the high cardiovascular risk in this population, particularly for those who cannot tolerate colchicine, the trial aims to determine whether monthly subcutaneous ziltivekimab 15 mg treatment can reduce MACE. Secondary endpoints assess kidney function decline, dialysis initiation, and mortality related to cardiovascular or renal causes. With more than 6,200 participants and a target of 1,044 primary outcomes, the ZEUS trial is powered to detect a 20% relative risk reduction in MACE. This study builds on prior evidence linking IL-6 to atherogenesis and inflammation, potentially establishing ziltivekimab as a novel anti-inflammatory strategy for high-risk patients.

The SOLID-TIMI 52 trial evaluated darapladib, a selective inhibitor of lipoprotein-associated phospholipase A2, hypothesizing that reducing plaque necrosis would lower cardiovascular risk. Despite its mechanistic rationale,

**Table 1. Comprehensive overview of significant clinical trials exploring the relationship between anti-inflammatory treatments and cardiovascular disease**

| Trial name | Description | Key findings |
|---|---|---|
| CIRT | Investigated low-dose methotrexate for cardiovascular outcomes in patients with prior myocardial infarction or coronary artery disease and metabolic conditions. | Methotrexate did not significantly reduce inflammatory markers or cardiovascular events compared to placebo. The final primary endpoint occurrence was similar between the methotrexate and placebo groups. |
| LODOCO | Assessed the efficacy of low-dose colchicine in reducing cardiovascular events post-myocardial infarction. | Significant reduction in MACE with colchicine treatment compared to placebo, highlighting its potential in reducing future cardiovascular incidents. |
| COLCOT | Evaluated colchicine's role in post-myocardial infarction care, focusing on a broad spectrum of cardiovascular outcomes. | Demonstrated a robust reduction in cardiovascular events, advocating for colchicine integration into post-myocardial infarction therapeutic regimens. |
| CANTOS | Explored the effects of canakinumab on patients with a history of myocardial infarction and a persistent inflammatory response. | Canakinumab significantly reduced the risk of cardiovascular events, particularly at the 150 mg dose, independent of lipid-lowering effects, suggesting benefits in cardiovascular-specific morbidity and mortality. |
| ZEUS | Evaluate ziltivekimab, an IL-6 inhibitor, for reducing inflammation and MACE in patients with CKD and ASCVD with elevated hsCRP levels. | The trial is enrolling >6,200 participants to assess whether ziltivekimab 15 mg monthly reduces MACE by 20%. Secondary endpoints include kidney function decline and cardiovascular or renal mortality. Expected completion in 2026. |
| SOLID-TIMI 52 | Investigated darapladib's role in stabilizing atherosclerotic plaques post-acute coronary syndrome. | No significant reduction in primary or secondary endpoints, indicating that darapladib did not confer significant cardiovascular protection post-acute coronary syndrome. |
| Latitude-TIMI 60 | Assessed the efficacy and safety of losmapimod in patients with acute myocardial infarction. | Despite reducing early inflammatory markers, losmapimod did not significantly improve long-term clinical outcomes, challenging the efficacy of p38 MAPK inhibition in post-myocardial infarction recovery. |

Abbreviations: ASCVD: Atherosclerotic cardiovascular disease; CKD: Chronic kidney disease; IL: Interleukin; MACE: Major adverse cardiovascular events; MAPK: Mitogen-activated protein kinase.

darapladib failed to improve outcomes, with an HR of 1.00 for major coronary events and 0.99 for cardiovascular death, myocardial infarction, or stroke.[26] Similarly, the LATITUDE-TIMI 60 trial investigated losmapimod, a p38 mitogen-activated protein kinase (MAPK) inhibitor, in acute coronary syndrome patients. While losmapimod reduced inflammatory marker levels, it did not translate into improved cardiovascular outcomes (HR: 0.99; 95% CI: 0.91 – 1.08), reinforcing the challenges of targeting single inflammatory pathways in atherosclerosis.[27]

## 8. Newer targets in IL signaling

ILs are cytokines produced by leukocytes that regulate immune responses by modulating inflammation, cell proliferation, and differentiation. They bind to specific receptors, triggering signaling cascades, such as the Janus kinase signal transducer and activator of transcription (JAK/STAT), MAPK, and NF-κB, influencing immune cell function and inflammatory gene expression.[28] Their dual ability to activate or suppress immune responses makes them central to autoimmune diseases, cancers, and cardiovascular pathologies. Dysregulation of IL signaling can lead to chronic inflammation and contribute to disease progression, making them attractive therapeutic targets.

The JAK/STAT pathway is pivotal in converting IL signals into gene expression changes that drive immune processes. Upon receptor binding, ILs activate JAK kinases, leading to STAT phosphorylation and nuclear translocation, where they regulate genes involved in inflammation and immune cell differentiation.[29] IL-6 and IL-10, for example, modulate both pro- and anti-inflammatory responses through this pathway. Dysregulation of the JAK/STAT pathway contributes to autoimmune disorders and malignancies, making it a key target for therapeutic intervention. The MAPK pathway, activated by ILs, governs cell proliferation, apoptosis, and cytokine production, influencing immune responses in acute and chronic inflammation.[30] Meanwhile, the NF-κB pathway, critical in immune modulation, is triggered by cytokines such as IL-1 and TNF. Upon activation, NF-κB translocates to the nucleus, promoting the transcription of pro-inflammatory genes essential for immune defense but also implicated in chronic inflammatory diseases, such as atherosclerosis.[31]

Several ILs play crucial roles in cardiovascular inflammation. IL-1, IL-6, and IL-18 contribute to vascular damage and plaque instability, accelerating atherosclerosis. Emerging cytokines, such as IL-17F, IL-33, IL-34, and

IL-36, offer new insights into vascular inflammation. IL-17F amplifies inflammatory cytokine and chemokine production, promoting neutrophil recruitment and endothelial dysfunction.[32] IL-33, released upon cellular injury, interacts with the ST2 receptor to activate myeloid differentiation primary response protein 88 and NF-κB, triggering pro-inflammatory cascades that drive immune cell recruitment.[33] IL-34 binds to macrophage colony-stimulating factor 1 receptor, sustaining monocyte and macrophage survival in inflamed tissues and exacerbating vascular pathology.[34] IL-36, a member of the IL-1 family, is activated by neutrophil-derived enzymes, amplifying immune cell adhesion and endothelial dysfunction, perpetuating chronic inflammation in vascular tissues.[35]

Advancements in monoclonal antibody therapies have provided targeted approaches to modulating vascular inflammation. In the CANTOS trial, canakinumab, an IL-1β inhibitor, demonstrated a 15% reduction in major cardiovascular events, validating IL-1β as a therapeutic target in atherosclerosis. Tocilizumab, an IL-6 receptor antagonist, improved myocardial salvage and significantly reduced inflammatory markers in the Assessing the Effect of Anti-IL-6 Treatment in Myocardial Infarction (ASSAIL-MI) trial, suggesting a role in acute coronary syndromes.[35,36] Secukinumab, targeting IL-17A, and ustekinumab, inhibiting IL-12/23, have demonstrated robust anti-inflammatory effects in autoimmune diseases and may provide cardiovascular benefits by reducing systemic inflammation.[37,38] Bimekizumab, which inhibits both IL-17A and IL-17F, showed a 91% reduction in the Psoriasis Area and Severity Index response rate in the BE READY trial, underscoring its potent anti-inflammatory properties.[39] Spesolimab, an IL-36 receptor antagonist, and astegolimab targeting IL-33/ST2 offer promising strategies to reduce endothelial activation and immune-driven vascular injury.[40,41]

## 9. Emerging therapeutic targets in CVD

Cluster of differentiation 47 (CD47), a transmembrane glycoprotein known as the "don't eat me" signal, prevents the clearance of apoptotic and damaged cells by macrophages, leading to persistent inflammation in atherosclerotic plaques. Inhibition of CD47-signal regulatory protein-alpha (SIRPα) signaling enhances macrophage-mediated efferocytosis, reducing necrotic core size, inflammation levels, and plaque burden.[42] Experimental studies have shown that CD47 blockade with monoclonal antibodies or SIRPα-Fc fusion proteins facilitates the clearance of apoptotic debris while improving endothelial function and neovascularization.[42] However, since CD47 inhibition also plays a role in immune surveillance, careful consideration is required to balance its cardiovascular benefits with potential risks, such as unintended immunosuppression and thrombosis.[42] Dual targeting of CD47 and vascular endothelial growth factor has been explored to enhance the resolution of inflammation while preventing excessive angiogenesis within unstable plaques.

Serum/glucocorticoid-regulated kinase 1 (SGK1), a kinase that regulates sodium retention, fibrosis, and cellular survival, is pivotal in cardiovascular remodeling and arrhythmogenesis. Upregulation of SGK1 has been implicated in myocardial hypertrophy, ischemia-reperfusion injury, and ion channel dysfunction.[43] Pharmacologic inhibition of SGK1 with small-molecule inhibitors, such as GSK650394 and EMD638683, has shown promise in mitigating hypertrophic signaling and reducing cardiac fibrosis. In addition to its role in cardiac remodeling, SGK1 inhibition has been explored as a potential strategy to modulate arrhythmogenic substrates by influencing $NaV_{1.5}$ and $K^+$ channel activity, reducing the likelihood of prolonged action potentials and arrhythmias. Moreover, SGK1 inhibition may provide cardioprotective effects against oxidative stress, particularly in conditions such as doxorubicin-induced cardiomyopathy.

P-selectin, an adhesion molecule stored in platelet α-granules and endothelial Weibel–Palade bodies, is a key driver of thromboinflammation. P-selectin contributes to platelet aggregation, endothelial dysfunction, and vascular inflammation by mediating leukocyte rolling and adhesion. Pharmacologic strategies targeting P-selectin include monoclonal antibodies, glycomimetic inhibitors, and small-molecule antagonists.[44] Crizanlizumab, a monoclonal antibody targeting P-selectin, has demonstrated clinical efficacy in reducing vaso-occlusive episodes in sickle cell disease and is now being investigated for cardiovascular applications to mitigate thrombosis-related complications.[44] In addition, PSI-697, an orally available P-selectin inhibitor, has shown potential in reducing atherogenesis and vascular injury in preclinical models.[44]

Growth/differentiation factor 15 (GDF-15), a member of the transforming growth factor-beta superfamily, has gained attention both as a biomarker and a therapeutic target in CVD. Elevated GDF-15 levels correlate with adverse outcomes in heart failure, myocardial infarction, and atrial fibrillation, reflecting its role in cellular stress responses and inflammation.[45] Therapeutically, modulation of GDF-15 signaling has been explored to mitigate maladaptive inflammatory responses and vascular dysfunction. GDF-15 influences leukocyte integrin activation and endothelial function, suggesting that targeting its signaling axis could provide cardioprotective effects, particularly in conditions characterized by excessive vascular inflammation and

remodeling.[45] Although GDF-15's role in metabolic regulation has made it a target in obesity and diabetes, its application in CVD remains an area of active investigation.

## 10. Conclusion

The therapeutic intervention of CVD has evolved, recognizing inflammation as a critical driver of disease progression and treatment response. While statins remain central to lipid-lowering therapy, advances in gene-based treatments, triglyceride-targeting agents, and novel anti-inflammatory strategies have expanded therapeutic options. Future research should prioritize optimizing combination therapies, refining patient selection, and addressing residual inflammatory risk to improve cardiovascular outcomes.

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REVIEW ARTICLE

# Electrical stimulation: Biological insights and therapeutic applications

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## Abstract

Electrical stimulation (ES) has emerged as a versatile modality in biomedical research, primarily due to its ability to modulate cellular activities across the cell membrane. As a barrier to electrical signals, the cell membrane plays a crucial role in healing, tissue regeneration, and cancer treatment. This review highlights the cellular processes involved in ES, focusing on the changes within and across the membrane, including effects on proteins, ions, and signaling pathways. ES regulates membrane potential and ion flow, such as calcium and sodium ions, which are essential for intercellular signaling and cell survival. In addition, ES facilitates electroporation, enhancing membrane permeability to allow controlled drug and gene delivery. It also modulates receptor sensitivity and cellular signaling efficiency by altering the lipid bilayer configuration and protein conformation. The applications of ES are extensive, including its use in wound healing, nerve regeneration, and as an adjunct to cancer treatments. Bioelectric Meridian Therapy, a new approach, employs ES for pain control, tissue repair stimulation, and energy flow regulation. Nevertheless, ES faces limitations such as heterogeneity in cellular responses, challenges in determining optimal stimulus parameters, and concerns regarding long-term safety. To address these issues, there is a need for real-time adaptive systems and personalized ES protocols to achieve safety and efficacy in all therapeutic settings. A deeper understanding of ES–cell membrane interactions can improve the current therapeutic paradigm and enhance the effectiveness of ES- based treatments. Further research is necessary to establish patient-specific ES parameters and integrate them into the precision medicine frameworks, minimizing side effects and improving treatment outcomes.

***Keywords:*** Neuromodulation; Electroceutical; Bioelectric therapy; Membrane dynamics; Electrical field applications

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## 1. Introduction

Electrical stimulation (ES) is an established area of biomedical research that plays a significant role in regulating cellular functions and enhancing treatment efficacy across various fields. ES leverages the body's electrical properties, such as the ability to promote wound healing and nerve regeneration, to modulate cellular activity.[1] It involves the use of controlled electric currents to influence cellular behavior, including movement, proliferation, and differentiation.[2] A central question in this field is how ES communicates with cellular membranes, which serve as the first line of contact for electrical signals and play a key role in determining cellular responses.[3]

### 1.1. Background on ES

Researchers have consistently shown much interest in the cellular mechanisms that drive physical changes in the body. One of the oldest approaches is ES, which facilitates cell regeneration and wound healing. ES has evolved over the years and is used in various fields, including tissue engineering, cancer therapy, and neurological intervention.[4-6] It has been shown to control a process known as galvanotaxis, a phenomenon where cells migrate directionally in response to an electric field of stem and fibroblast cells, which is critical in wound healing and tissue regeneration.[7] Cell membranes primarily determine the ability of ES to modulate cellular responses, as they regulate the transport of ions and molecules and play a critical role in signal transduction pathways (Figure 1).

### 1.2. Role of the cell membrane in ES

The cell membrane is sensitive to extracellular electrical stimuli due to its composition and organization. The lipid bilayer consists of proteins, ions, and receptors that regulate the transfer of charged materials into and out of the cell.[8] This regulation is needed to control the concentrations of the ions inside the cell and establish the membrane potential – the voltage across a membrane – which is very important in many cellular activities. When applied, ES can alter the membrane potential, impacting the signaling function and regulating required cellular behaviors, such as division and apoptosis.[9] Ion channels within the membrane respond to electrical currents, enabling the controlled influx and efflux of ions, such as calcium ($Ca^{2+}$), sodium ($Na^{+}$), and potassium ($K^{+}$), which in turn trigger downstream cellular processes.[10]

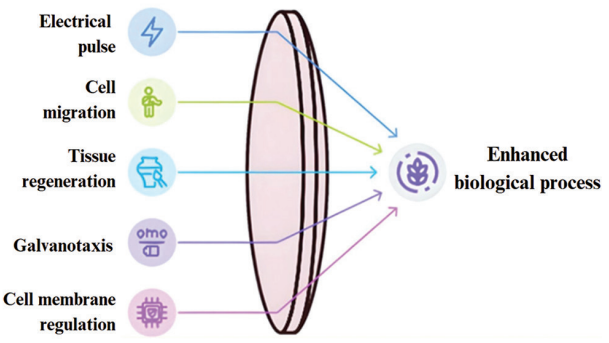


**Figure 1.** Effects of electrical signals on cellular processes

Bioelectric meridian therapy (BMT) is a relatively recent therapy modality that relies on the theoretical framework of ES. By applying low electric current at specific points along the body 's meridian pathways, BMT can effectively relieve muscle tension, remodel damaged cellular tissue, and reinstate the energy flows along the meridians. This therapy is based on the traditional Chinese medicine concept of meridians, which are believed to be pathways that conduct energy throughout the body.[11]

Depending on its impact on the cell membrane, through which signals are received, the effectiveness of ES as a treatment is closely tied to how the membrane responds. Many studies have indicated that the electrical charge of the cell membrane affects the ion channels and the membrane-bound receptors necessary for growth and repair processes.[12] ES can thereby modulate these pathways to improve tissue remodeling and facilitate regeneration. For instance, there is evidence that ES facilitates axonal regeneration in various neural tissues and enhances nerve functional recovery in peripheral nerve injury.[13] ES activates calcium signaling in muscle cells, which are critical for muscle contraction and injury healing, making ES beneficial for rehabilitation.[14]

One of the key goals of current ES research is to describe how ES influences specific cellular structures, especially the cell membrane. Understanding how the cell membrane regulates the effects of ES may reveal new therapeutic applications of ES— for example, in suppressing cancer cell growth in oncology or directing stem cell differentiation in tissue engineering.[15] By advancing the knowledge of these mechanisms, researchers aim to develop optimized ES protocols and parameters to achieve better therapeutic goals (Figure 2).

The potential applications of ES are extensive, with new facets of bioelectricity and its effects on living organisms continually being uncovered. I n regenerative medicine, ES has been shown to increase the viability of cells responsible for tissue repair, such as fibroblasts and osteoblasts, and to accelerate healing rates.[16] ES is also being explored for its role in inflammation management. It can influence immune cell behavior at sites of chronic diseases, such as arthritis and chronic ulcers.[17] These anti-inflammatory properties have made ES a valuable non-invasive tool

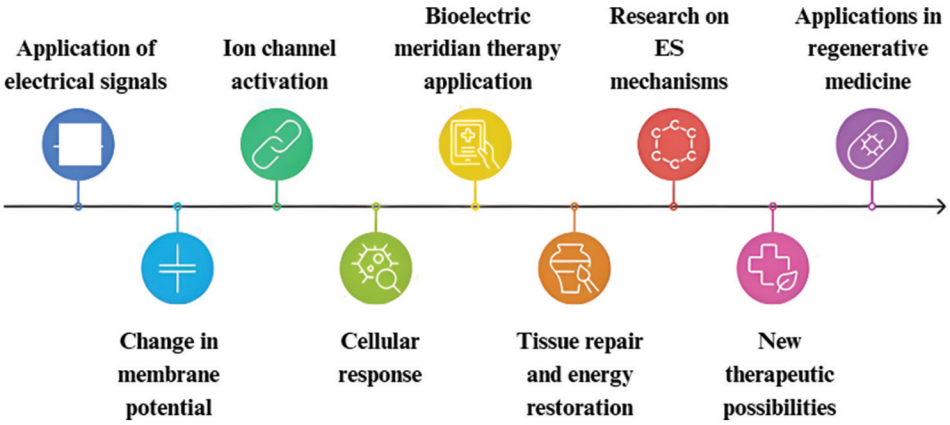


**Figure 2.** Role of electrical signals in cell membrane function
Abbreviation: ES: Electrical stimulation.

for managing chronic inflammatory conditions and promoting post-surgical recovery.

### 1.3. Review scope

This review highlights the role of ES in inducing cellular changes, with a primary focus on the cell membrane as the interface for bioelectric signals. Under the influence of ES, the cell membrane plays a pivotal role in mediating ion dynamics, receptor modulation, and signaling pathways.

In addition to cellular-level effects, the review incorporates insights into therapeutic applications that extend beyond the cell membrane. These include techniques such as BMT and deep brain stimulation, which leverage the broader electrical properties of tissues and organs for clinical benefits. The inclusion of such applications demonstrates the translational potential of ES in areas, such as pain management, regenerative medicine, and neurological retraining.[18]

In addition, this review also highlights emerging technologies, such as functional ES and spinal cord stimulation, which further bridge the gap between fundamental biological insights and therapeutic innovations. These multidisciplinary approaches underscore the significance of integrating cell membrane dynamics with broader clinical applications.

With the growth of the bioelectricity field, ES has increasingly been integrated into clinical practices, playing a crucial role in addressing current medical challenges. This study contributes to clarifying the role of the cell membrane in ES response, bringing the concept of bioelectric-based therapy closer to clinical reality. Such advancements suggest that treatments could eventually be tailored to a patient's natural electrochemical signaling patterns. Ongoing research on ES and its interaction with the cell membrane holds great promise in discoveries that will be relevant to both scientists and clinicians in bioelectric medicine. Increasing the efficacy of accessible techniques, such as BMT or developing new approaches to address complex illnesses through ES can significantly impact the future of healthcare policy and practice.

## 2. Mechanisms of ES on the cell membrane

ES is a biophysical technique that utilizes applied electric fields to activate several cellular responses. This interaction involves the cell membrane, which is made of a non-selectively permeable lipid bilayer divided into two by a selectively permeable barrier. The embedded proteins regulate ion flow and facilitate cellular communication and interaction. ES influences membrane structure and function at several levels and regulates many processes, ranging from neuronal communication to cell remodeling.

### 2.1. Membrane potential and ion dynamics

The electrical potential across a membrane at rest determines cellular functions. This potential arises from differences in the intracellular and extracellular ion concentrations, which are regulated by ion channels, pumps, and exchangers. When ES is applied, it can initiate specific ion-related changes depending on the characteristics of the stimulus.

#### *2.1.1. Depolarization and hyperpolarization*

ES affects the membrane potential by applying an external electrical field. Depending on the nature and strength of the stimulus, the cell may undergo depolarization, where the membrane potential becomes less negative, or hyperpolarization, where the membrane potential becomes more negative. This alteration is significant in excitable cells, such as neurons and myocytes. They can also trigger action potential or alter synaptic transmission.[19]

#### *2.1.2. Ionic flux*

Voltage-gated ion channels play a key role in the cellular response to ES by allowing ions such as $Na^+$, $K^+$, and $Ca^{2+}$ to move across the membrane. Among these, voltage-gated calcium channels are particularly important, as the influx of $Ca^{2+}$ into the cells is essential for intracellular signaling, neurotransmitter secretion, and gene expression. This calcium entry is generally coupled with changes in intracellular $Ca^{2+}$ stores and $Ca^{2+}$ signaling, which influences cell viability and renewal.[20]

### 2.2. Permeability modulation

The electric current affects the structural and functional characteristics of the lipid bilayer and changes membrane permeability. This phenomenon is crucial in drug delivery systems, gene delivery, and cancer therapies.

#### *2.2.1. Electroporation*

Electroporation forms nanopores with the help of high electrical pulses to temporarily destabilize the membrane integrity. These pores permit molecules such as DNA, RNA, and drugs to diffuse passively into the cells. This technique has significantly transformed molecular medicine, particularly in applications such as gene editing and immunotherapy.[21,22]

#### *2.2.2. Lipid bilayer dynamics*

ES may alter the arrangement of lipids in the bilayer, affecting its fluidity and phase behavior. This reorganization affects membrane stability and the distribution of signaling molecules, such as the formation of lipid rafts. Such changes can control receptor aggregation and signal amplification.[23]

### 2.3. Membrane protein and receptor impact

Transport proteins, receptors, and ion channels are proteins embedded in the cell membrane. They have diverse biochemical functions, including signal transduction. ES plays a significant role in the structure and coupling constitution of these proteins.

ES creates an electrical field that alters the conformational state of voltage-sensitive membrane proteins. For instance, voltage-sensitive phosphatases change conformation to regulate the activity of the phosphatases and signaling pathways.[24]

Moreover, ES enhances the functional competence and receptiveness of the receptors on the cell membrane. Due to increased clustering and redistribution, growth factor receptors and neurotransmitters become more responsive, leading to enhanced cellular activation. This effect has been applied in nerve repair and wound healing treatments.[25] A recent case report demonstrated the successful application of this approach in treating post-COVID-19 symptoms effectively. Patients undergoing therapy gained restored nerve functioning, muscle strength, and cognition, and an overall enhancement in well-being, attributed to improved bio-energy circulation. The findings highlight the necessity to investigate the effectiveness of BMT as a therapeutic approach for viral disease recovery.[26]

In terms of enhancing protein–ligand binding, ES can improve the binding constants of ligands to their receptors by altering the local membrane conditions. This mechanism is critical in therapy that involves targeted drug delivery and tissue engineering.[27]

The extent of ES effects on cell membrane potentials is deemed a promising therapeutic and biological phenomenon in medicine and biotechnology. In neurorehabilitation, ES is used to restore action potential and synaptic plasticity. It assists patients with spinal cord injuries, brain injuries, and stroke. Furthermore, it renews the axons, fortifies neural connections, and encourages functional gain by stimulating neurotrophic elements and significant pathways.[16] Including ES in rehabilitation programs enhances motor activity and decreases the muscle tone of the affected patient.

In addition, ES also activates the fibroblast and endothelial cells in tissue remodeling, promoting wound healing and new blood vessel formation. It also stimulates osteoblast proliferation and bone remodeling, facilitating the development of next-generation bioelectric scaffolds for tissue regeneration.[1] In oncology, electroporation-based therapies apply ES to increase drug uptake into cancer cells, augmenting the effect of treatments such as electrochemotherapy and reducing systemic side effects.[28] In addition, ES also enhances cancer immunotherapy by facilitating the delivery of genes that stimulate immune responses in tumors.

ES is very effective in treating cardiac and muscular disorders (Figure 3). Devices such as pacemakers and defibrillators help regulate heart rhythms in patients with arrhythmias or heart failure by synchronizing cardiac activity. Similarly, neuromuscular electrical stimulators are used to treat muscle atrophy and improve patient mobility.[29] Recent developments and growing insights into the molecular mechanisms of ES suggest that its clinical applications will continue to expand in the near future.

## 3. Biological effects of ES on cellular processes

ES has emerged as a complex, multifaceted technique with broad applications in cell biology, regenerative medicine, and tissue engineering. By regulating different cellular functions at or across the cellular signaling level,

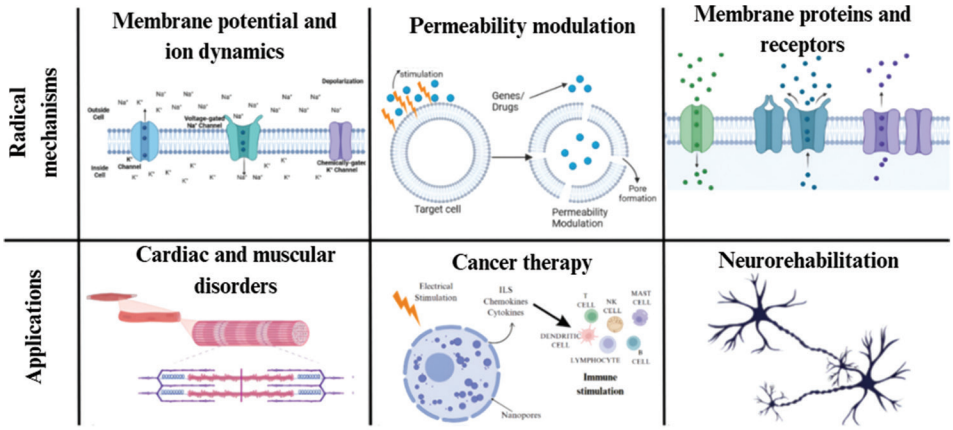


**Figure 3.** Mechanisms of action and clinical applications of electrical stimulation

ES contributed to improved tissue remodeling, repair, and therapeutic outcomes. Recent studies have expanded our understanding of these processes, integrating established and emerging insights into ES's mechanism of action.

### 3.1. Activation of signaling pathways

ES activates intracellular signaling by modulating the electrical and chemical milieu of the cells. Based on the signals from applied electric fields, voltage-gated ion channels allow $Ca^{2+}$ to enter the cells and initiate a sequence of intracellular signals. Recent research stresses that specific ion channels, including aquaporins, mediate ES's effects. For example, alternating current stimulation increases the number of open aquaporin channels without altering the membrane potential, providing evidence for a selective mechanism of cell hydration and ion transport.[30]

Furthermore, ES activates the mitogen-activated protein kinase/extracellular signal-regulated kinase and phosphatidylinositol 3 kinase/protein kinase B for cell survival and proliferation. Studies using pulsed electromagnetic field stimulation have shown the possibility of modulating oxidative stress by reducing reactive oxygen species levels and increasing antioxidant markers such as superoxide dismutase. This dual effect contributes to maintaining cellular stability in physiological and therapeutic states.[31] Recent research also shows that ES plays a role in angiogenesis and inflammation by regulating transcription factors such as hypoxia-inducible factor 1-alpha and nuclear factor kappa beta. These pathways enhance cellular metabolism and repair. Notably, the ability of ES to target hypoxic or damaged cells suggests its potential utility in treating ischemia or chronic wounds.

### 3.2. Cell adhesion and migration

Cell adhesion and migration are essential in various processes, such as wound healing, immune response, and tissue remodeling.[32] E S enhances these processes by modulating the interaction with adhesion molecules and regulating the cytoskeletal rearrangements. Studies involving low-amplitude direct current fields suggest that increased receptor proteins and focal adhesion kinase proteins are necessary for cell adhesion and migration into the extracellular matrix.[4]

The movement of cells in response to ES is referred to as galvanotaxis or electrotaxis. When the field strength is optimized, various cell types, such as keratinocytes, fibroblasts, and even immune cells, exhibit directed migration toward the cathode. These observations are derived from studies using capacitive coupling systems, which create homogenous electric fields. Such stimulation promotes cell migration and increases the deposition of glycosaminoglycans and extracellular matrix components.[33] These findings are clinically valuable, offering promising strategies for improving tissue repair.

### 3.3. Influence on growth and differentiation

ES modulates stem cell behavior and fate by recreating physiological electrical signals. For instance, biphasic waveforms commonly used in tissue engineering of the neural system enhance the differentiation of neural stem cells into neurons with increased neurite length and branching points. Conductive scaffolds improve this impact by creating favorable conditions for neural regrowth.[34] Similarly, mesenchymal stem cells undergoing pulsed ES have a higher osteogenic differentiation outcome. Previous studies have shown that runt-related transcription factor 2 and osteopontin as essential biomarkers that are upregulated during this process.[35] Furthermore, culturing cells with biochemical signals such as sulfated hyaluronan, combined with pulsed electromagnetic field stimulation, can boost bone matrix development, offering a novel strategy for orthopedic medicine.[36]

Emerging applications of ES also include soft tissue engineering involving myogenic and chondrogenic differentiation. In these cases, specific electrical parameters, such as frequency and amplitude, are essential to obtain specific results. For instance, low-frequency stimulation (less than 1 kHz) promotes cartilage matrix deposition, while higher frequencies support muscle regeneration.[4]

### 3.4. Optimization of ES parameters

ES is known to be sensitive to the waveform, intensity (typically ranging from 0.1 mA to 10 mA), pulse duration (ranging from 0.1 ms to 1 ms), frequency (from 1 Hz to 1,000 Hz), and polarity of the electrical field.[37] These parameters should be manipulated to achieve the best outcome where ES can activate the intended biological response while simultaneously alleviating the negative impacts. For instance, studies have shown that for skin wound recovery using surface electrodes, optimal parameter ranges include an intensity between 1 mA and 5 mA, a pulse duration of 100 ms to 300 ms, and a frequency between 10 Hz and 100 Hz.[38] Such targeted configurations can enhance tissue repair and reduce inflammation while avoiding thermal damage or oxidative stress.

The two key parameters include waveform type and electrical field strength. ES encompasses various techniques, including monophasic and biphasic stimulation, used to influence cellular activity through controlled electrical currents. Biphasic stimulation, a subset of ES, has emerged as a preferred approach in long-term biomedical applications. It is characterized by alternating polarity within each pulse, making it particularly effective in reducing adverse effects associated with continuous stimulation.

Biphasic stimulation has gained popularity, particularly for long-term applications, due to its ability to minimize charge accumulation at the electrodes.[39] Comparative studies indicate that biphasic stimulation typically operates with frequencies ranging from 10 Hz to 100 Hz and pulse durations in the range of a few microseconds to a few milliseconds per phase, while monophasic stimulation often employs higher currents over shorter durations, leading to increased risks of electrode fouling and tissue damage.[40] These differences make biphasic stimulation more favorable for maintaining electrode stability and promoting long-term cellular health.

Monophasic currents may result in charge deposited on the electrode surface and a higher likelihood of electrochemical side reactions through the production of hazardous products such as hydrogen and oxygen. In contrast, biphasic waveforms are characterized by a reversal of polarity per pulse. This characteristic reduces the likelihood of electrode fouling and prevents oxidative stress, which could damage the neighboring tissues.[41] This is particularly essential in applications requiring prolonged or regenerative uses, such as tissue engineering, where a constant stimulus is needed to modify cell behavior without causing cell death.

Monophasic stimulation is less safe for long-term studies because it can elicit an immediate cellular reaction, such as polarization or migration. It also exhibits high toxicity during long-term experiments. For example, high currents over long periods may result in thermal effects at specific sites where current enters and exits the tissue, potentially causing thermal damage to the cells.[42] However, recent advancements in electrode design have mitigated some of these issues. New findings highlight that improved electrode designs, such as platinum coating or conductive polymer applied to the sharpened tips of the electrodes, have reduced the effects of charge deposition and improved ES effectiveness. Furthermore, in the case of inductive or capacitive coupling, a non-invasive solution has been developed that eliminates the need to directly touch electrodes. These methods involve using electromagnetic fields or capacitive plates, transmitting electric fields to cells without the risks associated with invasive electrodes, which are less effective and more dangerous.[43] These advances also address challenges related to electrode stability and biocompatibility (Table 1).

### 3.5. Latest developments and future directions

ES is expected to shift from conventional procedures to complex techniques due to bioelectronics and real-time monitoring, especially from 2024. It is likely that the integration of intelligent systems, which will allow the stimulation parameters to be adapted based on cellular response, will dramatically evolve ES applications. These systems can assess the cellular response in real time, altering parameters such as current, frequency, and waveform to achieve the best therapeutic results. This approach may have the capacity to improve the accuracy and effectiveness of ES therapies, particularly in regenerative medicine, where differentiation responses depend on the tissue type.[44] Another rapidly advancing area is the design of conductive scaffolds at the nanoscale. These scaffolds are engineered to integrate with body tissues, providing mechanical support and bioelectric guidance to cells. The combination of ES with such advanced materials marks a new era in tissue engineering, particularly in the regeneration of bones, cartilage, and nerve tissues.[45]

In addition, the combination of ES with additional physical cues, such as mechanical stress and light, has provided new directions toward possible treatments.

**Table 1. Summary of the biological effects of electrical stimulation**

| Biological effect | Mechanism | Signaling pathway | Ion channels/ proteins | Cellular outcomes | Applications | Advancements | Optimization | References |
|---|---|---|---|---|---|---|---|---|
| Signaling pathway activation | Voltage-gated ion channels increase calcium ion influx. | MAPK/ERK, PI3K/Akt | Voltage-gated calcium ion channels | Reduces reactive oxygen species and increases antioxidants. | Tissue repair, chronic wound healing, ischemia | Improved oxidative stress management | Calcium ion channel activation, antioxidative effects | 30,31 |
| Adhesion and migration | Upregulates integrins and focal adhesion kinaseproteins. | Not specified | Integrins, focal adhesion kinase | Improves cell anchorage, migration, and extracellular matrix deposition. | Tissue regeneration, immune responses | Enhanced galvanotaxis | Extracellular matrix stimulation | 32,33 |
| Growth and differentiation | Promotes stem cell differentiation | Biochemical signals (e.g., bone morphogenetic protein ) | Osteoblast proteins | Improves neural regeneration, osteogenesis, and cartilage formation. | Neural repair, orthopedic medicine | Bone/cartilage scaffolding | Neural and tissue matrix optimization | 34,35,36 |
| Parameter optimization | Biphasic waveforms minimize oxidative stress. | Not specified | Capacitive coupling electrodes | Safe, sustained stimulation, minimal side effects. | Chronic stimulation, tissue engineering | Reduced electrode fouling | Focused waveforms | 39,41,42 |
| Emerging techniques | Real-time adaptive systems for precise control. | Multimodal pathways | Nano-scaffolds | Enhanced accuracy, biocompatibility, and regeneration. | Spinal injury repair, soft tissue engineering | Integration of nano-approaches | Adaptive methods for real-time control | 44,45,46 |

Abbreviations: MAPK/ERK: Mitogen-activated protein kinase/extracellular signal-regulated kinase; PI3K/Akt: Phosphatidylinositol 3-kinase/protein kinase B.

Recent research has focused on optimizing the duration and mechanical forces applied during ES to better mimic the physiological environment and enhance tissue regeneration efficacy. For example, applying mechanical loading in combination with electrical fields can encourage stem cells to differentiate into mature bone- forming cells called osteoblasts. Similarly, photo-biomodulation therapy leverages cellular responses to light, and when combined with ES, can simultaneously promote the reparative and regenerative processes.[46] These multimodal strategies hold significant promise for tissue engineering, especially in tissues that require complex cellular behavior to regain their functionality. The superposition of electrical fields, mechanical stimuli, and light signals presents a powerful approach for localized therapy. It may improve severe conditions, such as spinal cord trauma, articular cartilage pathology, and chronic non-healing ulcers.

## 4. Applications of ES in biomedical research

Recently, ES has emerged as an effective technique in biomedical studies due to its role in modulating cellular responses and functions. It has been used experimentally to investigate various treatment options by applying specific electrical currents to cells and tissues. These applications extend to various fields, such as wound healing and tissue engineering, drug and gene delivery, cancer treatment, and novel therapies, such as BMT, that may be useful in treating lymphedema. These areas demonstrate how ES could enhance treatments for various illnesses, offering patient-friendly alternatives for managing multiple pathophysiologic states.[47] ES has recently garnered significant attention for its application in wound healing. Non-healing ulcers, caused by diabetes or pressure injuries, often do not respond to conventional treatments and may benefit from adjuvant therapies, such as ES. Sarco-ES may contribute to enhanced cellular functions such as cellular migration, proliferation, and deposition of collagen filaments, which are essential in wound healing. Scientific evidence also shows that ES can activate fibroblasts and keratinocytes, two cells crucial for healing.[48] Moreover, it has been shown that ES induces the expression of growth factors, including transforming growth factor- beta and vascular endothelial growth factor, which are essential in tissue remodeling and neoangiogenesis. ES also promotes the release of anti-inflammatory cytokines, contributing to a more favorable healing environment at the wound site.[16] These discoveries have led to the recognition of ES as a possible modality for chronic, non-healing wounds as an additional treatment modality.

Recent advancements in ES have exhibited significant results for targeting drugs, DNA, or RNA in tissues. This is mainly achieved through electroporation, where

electrical impulses induce the opening of cellular components within the cell membrane, allowing large molecules to enter the cell.[49] This mechanism has important implications for gene therapy, as the efficient delivery of genetic material to target cells is essential for treating genetic diseases. Electroporation enhances gene transfer by increasing the uptake of DNA or RNA into cells, improving therapeutic outcomes. Electroporation also facilitates drug uptake, making it valuable for the delivery of chemotherapeutic agents, antiviral medications, and other drugs requiring targeted administration. By modifying the intensity, duration, and frequency of electrical pulses, the delivery process can be made more effective, allowing for greater precision and control in treatment applications.[50]

ES has also been reviewed as a complementary modality for use with conventional cancer treatments, including chemotherapy and radiation. ES may have potential for clinical application due to its ability to destabilize the membrane of cancer cells and trigger apoptosis. Several studies have noted that ES can increase the cytotoxic effect of chemotherapy and radiation on tumor cells by increasing membrane permeability.[5,51] This sensitization of cancer cells augments the effectiveness of standard cancer therapies. ES can also positively influence the tumor microenvironment by improving blood flow to tumor sites, enhancing the transport of oxygen-rich blood and other therapeutic agents required for effective cancer treatment.[52] In addition, transcutaneous electrical nerve stimulation has been used to manage cancer-related pain and enhance the quality of life of cancer patients undergoing intensive treatment regimes.[53] These promising results indicate that ES could serve as a valuable adjunct in comprehensive cancer therapy strategies.

BMT is a relatively novel use of ES, which has shown potential in treating lymphedema, a condition in which an abnormal buildup of lymphatic fluid in the limbs causes pain, swelling, and limitation of movement. BMT is based on the application of low-intensity ES to the affected area, aiming to enhance the lymphatic system's function by stimulating muscle contractions and activating lymphatic capillary beds.[54] The process promotes lymphatic stability and accelerates the removal of excess fluid from the tissues, thereby reducing swelling and improving mobility. Research has found that BMT can greatly enhance edema management compared to manual lymphatic drainage and compressions.[55] Compared to conventional approaches, BMT is less labor-intensive and offers more sustained control of lymphedema. Its adaptability for home-based care provides patients with a convenient therapy that reduces reliance on manual procedures.[14] Furthermore, BMT has fewer side effects and may be better suited for patients with chronic lymphedema, particularly when other treatments may not be as effective or difficult to administer.[54] Unlike many other therapies, BMT addresses both the physical and functional symptoms of lymphedema and has a strong potential to improve patient outcomes and quality of life.

## 5. Limitations and challenges

ES is increasingly recognized as a significant approach for modulating cellular processes, including proliferation, differentiation, and migration. However, several limitations and challenges were identified that render ES impracticable in real-life practice. One of the most significant challenges is the difficulty in eliciting an intended cellular response, as these responses vary depending on cell type, cell age, and environmental conditions. For example, fibroblasts, neurons, and endothelial cells may each respond differently to the same type of electrical input, making it difficult to predict the effects of ES across various biological contexts.[56] Moreover, factors such as disease states (e.g., cancer or diabetes) or certain culture conditions can further influence cellular responsiveness, adding to the uncertainty. Even within the same cell type, variability in samples can complicate efforts to standardize ES procedures. This inconsistency makes it difficult to compare results across studies and reduces the reproducibility of experiments.[9]

Another key challenge lies in the complexity of treatment parameters. The effectiveness of E S depends on four parameters: voltage, frequency, pulse duration, and waveform. However, there is no universally optimal combination of these parameters. For example, extremely low-frequency electrical fields in our natural environment can promote cell division in specific cell types, while high-frequency ES may be more effective in guiding differentiation or improving cell functions.[57] Despite the growing body of evidence, identifying a universally applicable stimulation regimen remains elusive, which complicates the development of standardized clinical protocols. In addition, non-optimal electrical factors such as oxidative stress, apoptosis, or inflammation, as described by Meng *et al.*,[58] can have a detrimental effect on the cellular level. This variability in cellular responses reinforces the notion that ES, while promising, requires meticulous parameter tuning in clinical settings, where precise outcomes are critical to patient safety and therapeutic success.

Furthermore, the following technical difficulties are observed as risks associated with ES and possible implications of its future use. While the short-term benefits of ES, such

as enhanced wound healing and tissue regeneration, are well-documented and supported by several studies,[59] the long-term impacts of prolonged ES application remain ambiguous. According to some researchers, electrical fields may deteriorate cells, alter gene function, or contribute to carcinogenic changes in some instances.[60] In addition, the systematic effects of ES on neighboring organs and tissues, particularly in *in vivo* models, have not been thoroughly explored. The lack of information on the procedure's long-term effects on tissues and cells presents a significant challenge to its safe implementation, especially in strategies that involve continuous stimulation over months or years, for chronic diseases or tissue regeneration. Safety concerns are further heightened by device failure, heat generation, electrical circuit malfunctions, and signal variability, all of which could cause adverse effects or therapeutic failure in clinical applications.[61] The examined device-related issues further complicate the use of ES in clinical practice since standard tools utilized for the interventions are sufficiently safe and comparable across studies (Table 2).

These three problems, the variability of cellular response, the absence of optimized stimulation parameters, and the uncertain side effects of ES, prove that ES is not a simple therapeutic approach. However, the field is still in its early stages. Further research is necessary to standardize the protocols for applying ES, optimize the parameters, and understand the long-term effects of ES on cells and tissues. Addressing these challenges will be essential for

**Table 2. Challenges and solutions in electrical stimulation applications**

| Issue | Challenges | Factors influencing outcomes | Examples/ implications | Current limitations | Potential risks | Proposed solutions | Research needs | References |
|---|---|---|---|---|---|---|---|---|
| Standardization difficulties | Variability across individual samples hampers the reproducibility and comparability of studies. | Sample variations, experimental setup differences | Difficulty comparing results across studies | No standardized experimental protocols | Limited reproducibility across studies | Create harmonized guidelines for experimental protocols | Conduct meta-analyses to harmonize methodologies | 9 |
| Variability in cellular responses | Cellular responses are not uniform across cell types, age, and environmental conditions. | Cell type, cell age, culture conditions | Difficult to predict effects on fibroblasts, neurons, and endothelial cells | Variability across studies and patient outcomes | Unpredictable therapeutic outcomes | Standardizing protocols through comprehensive studies | Further understanding of cell-specific responses | 56 |
| Difficulty in parameter optimization | Delicate voltage, frequency, pulse duration, and waveform tuning are required. | Voltage, frequency, pulse duration, waveform | Extremely low-frequency fields stimulate division; high-frequency fields induce differentiation | No universal optimal settings for stimulation parameters | Ineffective or harmful stimulation | Develop cell-type-specific protocols | Optimization of stimulation parameters | 57 |
| Non-optimal electrical factors | Adverse effects due to oxidative stress, apoptosis, or inflammation | Non-optimized parameters | Negative cellular responses | Limited understanding of adverse effects | Oxidative damage or inflammation | Enhance optimization algorithms for clinical applications | Study the mechanisms of adverse electrical stimulation effects | 58 |
| Safety and long-term effects | Limited knowledge of chronic effects; risks of degeneration, gene changes, or carcinogenesis | Prolonged stimulation, neighboring tissue impact | Tissue degeneration, inflammation, or potential cancer risks | Lack of systematic studies on chronic impacts | Potential carcinogenesis or tissue damage | Longitudinal studies to assess chronic safety impacts | Study chronic effects on various tissues | 60 |
| Device-related issues | Device failure, heat generation, electrical circuit issues, and signal fluctuations | Device design, reliability, and maintenance | Treatment failure or adverse effects in clinical applications | Insufficiently reliable and safe medical devices | Treatment failure or adverse side effects | Improve device reliability and implement rigorous safety testing | Development of robust and standardized devices | 61 |

developing ES from an experimental concept to a reliable model for clinical practice.

## 6. Future perspectives

The influence of ES on cell membranes has stimulated considerable interest in cell membrane-based therapeutic approaches. Further research development in this area presents several future trends with potential in clinical and molecular biology.

### 6.1. Advancements in ES technology

New developments have been achieved in ES techniques, particularly in enhancing parameter control for biological experiments and therapeutic applications. For example, transcranial direct current stimulation and deep brain stimulation have improved over the years in their ability to affect cell membranes in target tissues.[62] A more ambitious prospect is the integration of nanotechnology and bioelectronic interfaces, extending ES applications to even more localized modes. For example, improving the type of electrodes on the nanoscale and micro electromechanical systems could enhance the accuracy of ES, enabling more accurate engagement of specific cellular reactions.[63] Such progress is likely to pave the way for tailored treatments across different fields, such as neurological and cardiovascular sciences.

### 6.2. Personalized medicine

However, as individuals respond differently to ES, the notion of patient-tailored medicine is becoming increasingly relevant in ES-based therapies. A patient's characteristics, including genetic predisposition, type of tissue, and disease status, may affect how cells react to ES.[64] The current density and frequency should be adjusted with the client's distinctive features to improve the therapeutic outcomes and reduce adverse effects. This personalized approach to ES-based therapies holds promise for enhancing the treatment of conditions, such as chronic pain, neurodegenerative diseases, and wound healing.

### 6.3. Expanding molecular understanding

Although there is an increasing understanding of the impact of ES, the molecular basis remains an essential focus. Future studies are expected to extend the knowledge of the molecular mechanisms underlying ES effects, particularly on signaling pathways, gene expression in various cell types, and ion channel regulation. New possibilities involve identifying more effective interventions based on the specific molecular targets that regulate cellular responses to electric signals.[65] Detailed insights into molecular changes in cells exposed to ES are likely to emerge with the help of advanced technologies, such as genomics, proteomics, and single-cell RNA sequencing.[66] Applying these advances to clinical practice can potentially improve therapeutic outcomes while offering a better perspective of the cellular and molecular changes elicited by ES. The ongoing collaboration between biologists, engineers, and clinicians is not merely beneficial but critical for harnessing the full potential of ES technologies. Emphasizing this interdisciplinary collaboration highlights the crucial role each field plays in advancing ES technologies.

### 6.4. BMT

BMT has seen remarkable innovation in recent years. It is being applied in pain management, physical therapy, and areas, such as treating wounds and skin care.[67] It has been reported that it can enhance tissue repair more rapidly by stimulating fibroblast activity, promoting collagen

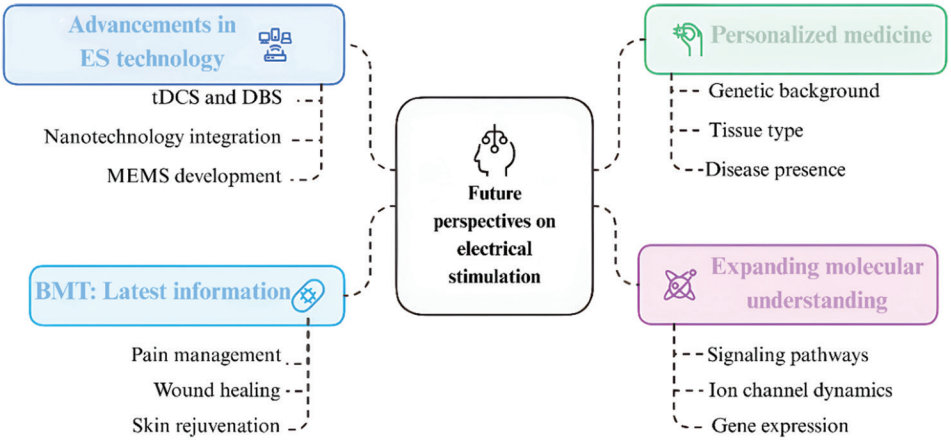


**Figure 4.** Future perspectives and applications of electrical stimulation
Abbreviations: BMT: Bioelectric Meridian Therapy; DBS: Deep brain stimulation; ES: Electrical stimulation; MEMS: Micro-electromechanical system; tDCS: Transcranial direct-current stimulation.

synthesis, and improving vasculature.[68] Recent clinical trials using BMT to treat chronic back pain and osteoarthritis in patients showed promising results, including pain reduction and improvement in patient mobility.

Furthermore, efforts are being made to combine BMT with other treatments, such as physiotherapy or acupuncture, to achieve better patient outcomes. When integrated with patient-specific exercises or manual therapy, BMT may enhance recovery rates and tissue performance.[69] Figure 4 illustrates the future perspectives and applications of ES in clinical and research fields, highlighting its advancements, personalized applications, and molecular understanding.

## 7. Conclusion

This review focuses on how ES has emerged as a unique research platform in the biomedical field, with novel therapeutic applications in wound healing, regenerative medicine, and oncology. The cell membrane plays a critical role in ES, acting as a flexible barrier that modulates cellular responses to electrical impulses. Electrical stimulus plays an essential role in regulating and controlling various biological activities, including cell signaling, cell movement, and specialization. These effects are achieved through changes in ion concentrations, alterations in the membrane permeability, and modifications to protein structures. ES applications have shown promise in improving wound healing, nerve regeneration, and cancer treatment through electroporation facilitated by BMT. The advancements highlighted in the literature emphasize ES's potential in alleviating pain, repairing tissues, and treating complicated ailments. However, several challenges remain, such as variability in cellular response, the optimization of measurement parameters, and concerns regarding long-term safety. To address these issues, further development of ES technologies is needed, especially by combining real-time adaptive systems and personal medicine. Modifying ES protocols based on the characteristics of each patient receiving the treatment and fine-tuning the stimulation parameters may also improve therapeutic efficacy and reduce adverse effects. As research continues to uncover the molecular and biophysical mechanisms underlying ES, the field may offer new approaches for numerous medical challenges and eventually shape the field of bioelectrical medicine—a potential new discipline, which could be recognized as "bioelectromediotronics."

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ACCSCIENCE PUBLISHING

Global Translational Medicine

REVIEW ARTICLE

# Evolution of tunneling techniques in periodontics: A narrative review

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## Abstract

Over the past several decades, a trend toward minimally invasive surgery has emerged in various disciplines of medicine and dentistry. In periodontics, one manifestation of this phenomenon is the use of tunneling techniques for mucoperiosteal flap reflection. Tunnel flaps are characterized by the establishment of a space between the alveolar bone or periosteum and the overlying soft tissue while maintaining intact interdental gingiva and/or crestal keratinized mucosa. The oral and facial interdental papillae and col areas remain intact during the procedure. Retaining interproximal tissue integrity may enhance wound stability during early healing, and limited evidence suggests that tunnel flaps can improve several patient-reported outcome measures, such as comfort level, initial esthetics, and time required for return to normal activities. Multiple refinements have been promulgated since the introduction of the first tunneling techniques, and clinical applications have expanded into numerous areas of the field, including surgical treatment of periodontitis, periodontal plastic surgery, and alveolar ridge augmentation. The purpose of this narrative review is to describe the evolution of tunneling techniques over time and suggest opportunities to further develop tunneling applications. Two clinical circumstances are described in which multi-surface tunneling at oral, facial, and proximal tooth surfaces can be employed to achieve favorable clinical and patient-oriented outcomes.

***Keywords:*** Alveolar bone loss; Gingival recession; Minimally invasive surgical procedures; Patient-reported outcome measures; Periodontitis; Tissue grafts

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## 1. Introduction

Although no universally accepted definition exists, minimally invasive surgery encompasses techniques that seek to accomplish surgical goals while limiting incision size, maximizing wound stability, and enhancing patient-oriented outcomes. Over the last several decades, virtually every surgical specialty of medicine and dentistry has

integrated minimally invasive techniques into the clinically accessible repertoire of procedures.[1] Multiple examples can be found in periodontics. Few studies have assessed clinical efficacy and long-term stability following laser periodontal therapy.[2] However, this minimally invasive procedure aims to provide definitive periodontitis treatment despite accessing intrabony defects (IBDs) through the gingival sulcus only, without any external incisions.[2] In contrast, the modified minimally invasive surgical technique (M-MIST) involves the use of limited incisions and minimal reflection of the defect-associated papilla to access the alveolar bone and root surface.[3] Long-term follow-up of a randomized controlled clinical trial demonstrated that IBDs treated with M-MIST alone or M-MIST and regenerative materials exhibited periodontal stability over a 10-year observation period.[3]

Tunnel flaps comprise a broad subset of minimally invasive techniques utilized within the field of periodontics (Figure 1). Derivations have been applied in periodontal plastic surgery, regenerative treatment of periodontitis, and alveolar ridge augmentation (ARA) (Table 1). The unifying feature of a tunnel flap is the avoidance of crestal incisions that establish distinct buccal/facial and palatal/lingual mucoperiosteal flaps. The interdental gingiva and crestal soft tissue at edentulous sites remain intact but may be freed from the alveolar bone. The surgeon may employ intrasulcular incisions only or add limited vertical vestibular incisions that do not extend to the gingival margin. Specialized suturing techniques or implantation of biomaterials may coronally position the tunnel flap. Irrespective of the procedure type, the rationale for selecting a tunnel flap usually includes enhancement of patient-reported outcome measures (PROMs), such as reduced post-operative swelling and discomfort, improved esthetics in the short term, and early return to normal activities.

Across all tunnel-based procedures in periodontics, the main limitation is the paucity of evidence confirming superior or equivalent clinical outcomes compared with procedures that rely on classic flap designs. Direct clinical comparisons between tunnel and conventional flaps are rare, and contradictory results appear in the literature. Nevertheless, outcomes achieved with tunnel-based periodontal surgeries are generally positive, and consistently, the procedures are well accepted by patients. The purpose of this report is to review the tunnel flaps that have been applied in periodontics and suggest further derivations of the technique.

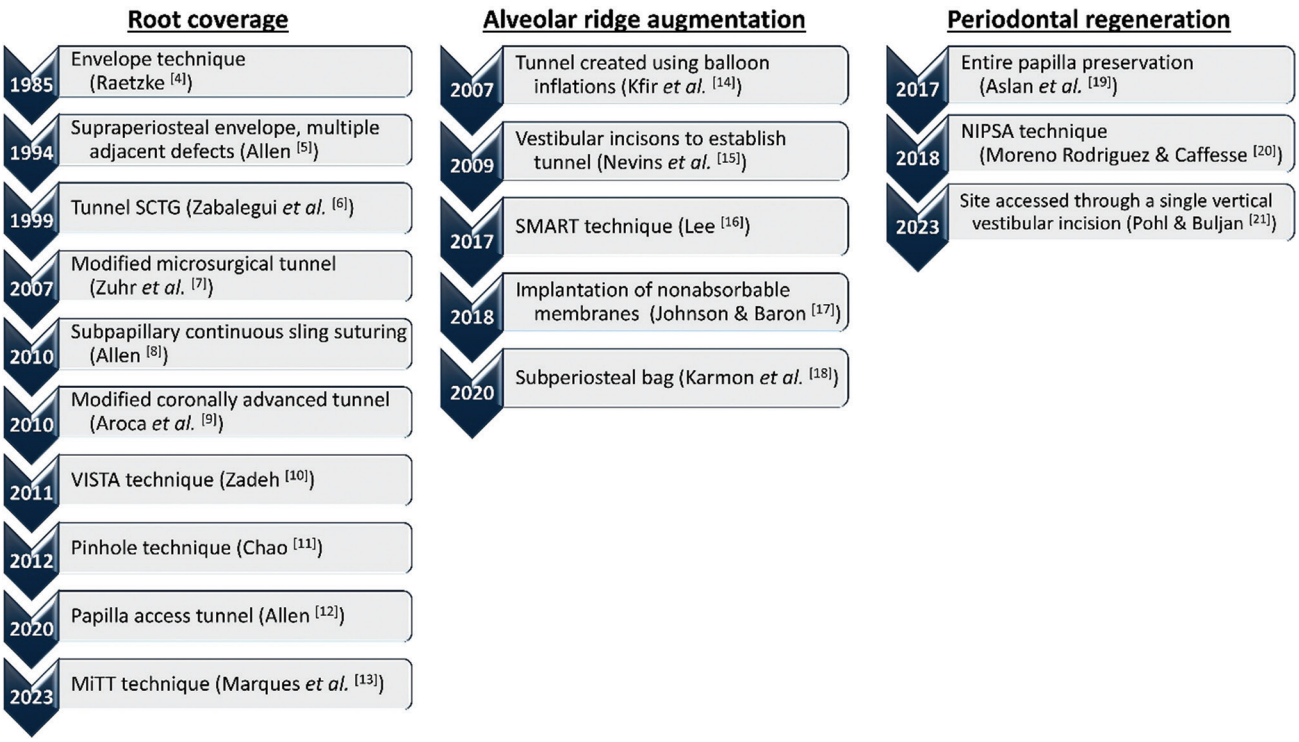


**Figure 1.** Evolution of tunnel flaps in periodontics for root coverage, alveolar ridge augmentation, and periodontal regeneration. Image created by the authors.

Abbreviations: MiTT: Mixed-thickness tunnel access; NIPSA: Non-incised papilla surgical approach; SCTG: Subepithelial connective tissue graft; SMART: Subperiosteal minimally invasive esthetic ridge augmentation technique; VISTA: Vestibular incision subperiosteal tunnel access.

**Table 1. Evolution of tunneling procedures in periodontics**

| Year | Authors | Procedure |
|---|---|---|
| | | **Periodontal plastic surgery** |
| 1985 | Raetzke[4] | Envelope technique. Applicable to isolated recession defects. |
| 1994 | Allen[5] | Supraperiosteal envelope. Applicable to single and multiple adjacent recession defects. Sharp dissection when tissue thickness permitted. |
| 1999 | Zabalegui *et al.*[6] | Tunnel subepithelial connective tissue graft. Partial-thickness dissection beyond the mucogingival junction. Use of sutures for graft positioning. |
| 2007 | Zuhr *et al.*[7] | Modified microsurgical tunnel technique. Use of specialized tunneling knives with sharp edges toward the periosteum. |
| 2010 | Allen[8] | Subpapillary continuous sling suturing method. Use of a single continuous suture to stabilize both the acellular dermal matrix and the overlying tunnel flap. |
| 2010 | Aroca *et al.*[9] | Modified coronally advanced tunnel. Composite stops at proximal contact areas to facilitate suturing. Use of a specialized knife-elevator instrument for tunnel preparation. Application of ethylenediaminetetraacetic acid and enamel matrix derivative. Papillae are freed from the interproximal alveolar crest. Mucoperiosteal dissection extended beyond the mucogingival junction. |
| 2011 | Zadeh[10] | Vestibular incision subperiosteal tunnel access (VISTA) technique. Use of vertical vestibular incisions to facilitate subperiosteal tunnel reflection. Use of polypropylene sutures bonded to the facial surfaces of the teeth. |
| 2012 | Chao[11] | The pinhole surgical technique. Use of specialized transmucosal papilla elevators to reflect full-thickness mucoperiosteal flaps through small incisions in the alveolar mucosa. Stabilization of marginal tissue in coronal position by implanting absorbable porcine collagen membrane strips. |
| 2020 | Allen[12] | Papilla access tunnel. Reflection of papilla to facilitate tunnel preparation in the presence of shallow recession defects and thin tissue. |
| 2023 | Marques *et al.*[13] | Mixed-thickness tunnel access. Vertical incisions terminating apical to the mucogingival junction. Split-thickness separation of the alveolar mucosa from the underlying muscles. Full-thickness tunnel flap reflected from the mucogingival junction to the gingival margin. Papilla tip intact. |
| | | **Alveolar ridge augmentation** |
| 2007 | Kfir *et al.*[14] | Use of a tunnel created using a series of balloon inflations for guided bone regeneration (GBR). |
| 2009 | Nevins *et al.*[15] | Tunneling technique for alveolar ridge augmentation involving vestibular incisions to create subperiosteal pouches into which recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and various biomaterials were implanted. No barrier membrane. |
| 2017 | Lee[16] | Subperiosteal minimally invasive esthetic ridge augmentation technique. Similar to the method described by Nevins *et al.*[15] Implantation of anorganic bovine bone mineral (ABBM) and rhPDGF-BB. No barrier membrane. |
| 2018 | Johnson and Baron[17] | Tunnel access for GBR in the maxillary anterior. Intrasulcular and vestibular incisions to facilitate subperiosteal tunnel preparation. Use of a dense polytetrafluoroethylene membrane and an allogeneic bone derivative. |
| 2020 | Karmon *et al.*[18] | GBR tunneling technique involving placement of a subperiosteal bag—a perforated, folded, and sutured collagen membrane filled with ABBM. |
| | | **Regenerative periodontal surgery** |
| 2017 | Aslan *et al.*[19] | Entire papilla preservation technique. A "tunnel-like" approach to papillae at intrabony defect (IBD) sites. |
| 2018 | Moreno Rodríguez and Caffesse[20] | Non-incised papilla surgical approach. Defect-associated papillae remain completely intact. IBDs are accessed through a single horizontal or oblique incision in the alveolar mucosa. |
| 2023 | Pohl and Buljan[21] | VISTA approach to periodontal regeneration. IBDs are accessed through a single vertical vestibular incision. |

## 2. Tunnel flaps in periodontal plastic surgery

### 2.1. Early tunnel flap techniques

Although not originally termed a tunnel flap, the "envelope" technique, introduced by Raetzke[4] in 1985, involved maintenance of the integrity of the interdental gingiva. In this technique, the marginal gingiva adjacent to the recession defect was excised to remove the sulcular epithelium, the root surface was scaled and planed, and citric acid was applied to condition the root. A partial thickness envelope was created, extending apically several millimeters beyond the gingival margin and laterally to the line angles of adjacent teeth. A small subepithelial connective tissue graft (SCTG) was harvested, tailored to the dimensions of the recipient site, implanted within the envelope, and stabilized with a tissue adhesive rather than sutures. After treating 12 recession defects in 10 patients,

Raetzke observed a mean gain in keratinized gingiva of 3.54 mm and a mean residual recession depth of 0.67 mm. Notably, the benefits of the technique that the author identified included PROMs commonly reported for tunnel-based root coverage procedures—minimal surgical trauma, favorable early healing, limited post-operative discomfort, and an esthetic appearance. Raetzke's technique has been described as elegantly simple, requiring neither external incisions nor sutures.[5] However, the technique's applicability was limited to isolated recession defects.[4]

Dr. Allen[5] presented the supraperiosteal envelope for root coverage procedures at isolated and multiple adjacent recession defects in 1994. At sites exhibiting gingiva of adequate thickness, sharp dissection was used to establish a supraperiostal envelope extending 3–5 mm lateral and apical to the recession defects. Full-thickness envelope preparation was used at sites presenting excessively thin gingiva. Allen advocated a uniform SCTG thickness of at least 1.5 mm. Placement of the SCTG within the envelope was accomplished using a temporary mattress suture to guide the graft into position while also using tissue forceps. Simple interrupted sutures at the mesial and distal graft margins introduced slight tension in the SCTG, and vertical mattress sutures in papilla areas stabilized the graft at the appropriate apicocoronal level.

In 1999, Zabalegui *et al.*[6] modified Raetzke's envelope technique—the tunnel SCTG—for the treatment of multiple adjacent recession defects. The authors described a "multi-envelope" recipient bed in which adjacent supraperiosteal envelopes were connected to form a tunnel. The partial thickness flap preparation established through the gingival sulcus extended to the mucogingival junction (MGJ) apically, and although the papilla tips remained intact, the base of the papillae was undermined using sharp dissection. To position and stabilize the SCTG, two sutures were introduced through the tunnel—one from the mesial aspect and the other from the distal. The suture needles entered the tunnel through attached gingiva lateral to the most mesial and distal recession defects and exited the largest or most central recession defect. The needles engaged the mesial and distal aspects of the graft, and then traveled back through the tunnel before emerging from the attached gingiva approximately 2 mm from the original insertion points. Gentle tension in the sutures pulled the SCTG into position, and after the sutures were tied, the graft was stabilized. Portions of the SCTG overlying the recession defects remained exposed, and no attempt was made to coronally advance facial flap margins to or beyond the cementoenamel junctions (CEJs). In a multi-center randomized trial, SCTG + tunnel and SCTG + coronally advanced flap (CAF) exhibited no significant difference in mean root coverage attained.[22] However, the tunnel procedure resulted in statistically greater gains in keratinized tissue width as well as less post-operative morbidity and pain.

Various modifications of the original tunnel technique have been proposed, including the use of specialized microsurgical instruments,[7] full-thickness elevation of the interproximal gingiva,[9] coronal advancement of the flap margin (Figure 2),[8,9,23] use of a biomaterial rather than an autologous graft,[8,9] and use of specialized suturing techniques.[8] In 2010, Dr. Allen[8] described a subpapillary continuous sling suturing technique for acellular dermal matrix (ADM) + tunnel flap. In this technique, a single continuous suture stabilized the ADM while coronally positioning the tunnel flap margin. A systematic review and meta-analysis found that when observations were limited to a single graft type (ADM or SCTG), CAF produced superior mean root coverage and complete root coverage (CRC) frequency compared with tunnel flaps.[23] Nevertheless, root coverage procedures that incorporate tunneling may yield superior patient-oriented outcomes.[7-9,22,23]

In the same year, Dr. Aroca *et al.*[24] introduced the modified coronally advanced tunnel (MCAT)—also called the coronally advanced modified tunnel—which included placement of "composite stops" at proximal contact areas to facilitate stabilizing the facial/buccal flap in a coronal position using sutures. The sulcular incisions and tunnel flap release were completed using a specialized knife-elevator instrument.[7] In addition, the root surfaces were chemically modified using ethylenediaminetetraacetic acid (EDTA), and enamel matrix derivative (EMD) was applied.[24] To permit coronal advancement of the tunnel flap, each papilla was freed from the interproximal alveolar crest, and the mucoperiosteal dissection extended apically beyond the MGJ.

Anatomic factors make gingival augmentation and root coverage at lingual recession defects in the mandibular anterior region uniquely challenging. This area presents the narrowest apicocoronal gingival width in the mouth, with an average measurement <3 mm.[25] Depending upon the proclination of the mandibular incisors, direct visualization can be extremely challenging intraoperatively, and the proximity of vital structures such as Wharton's duct adds additional complexity to the procedure.[26] A post-operative infection following this procedure type could involve the sublingual space, and by extension, the adjacent submandibular space and other deep fascial orofacial spaces.[27] For these reasons, risk-informed clinicians and patients may elect to defer treatment. Nevertheless, tunnel-based procedures may offer a more favorable risk profile when treating lingual

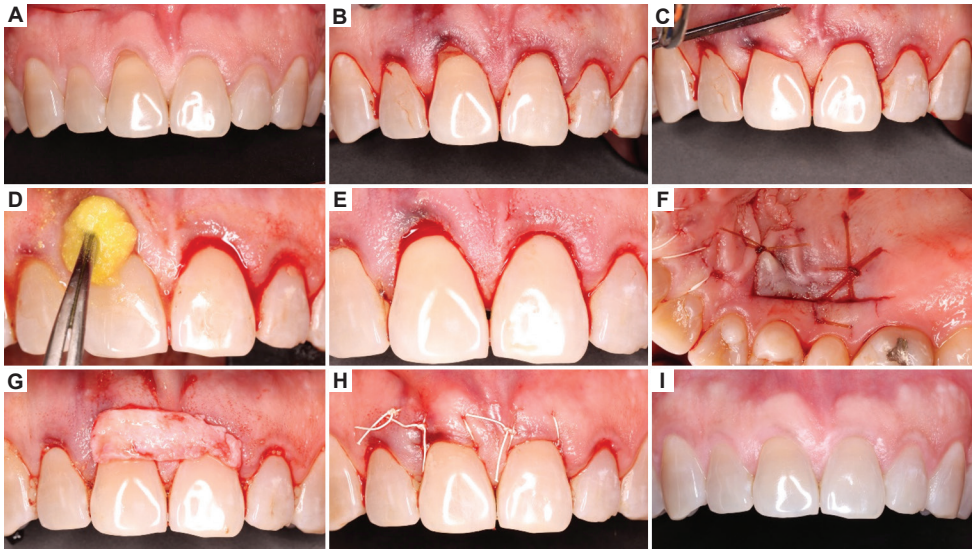


**Figure 2.** Coronally advanced tunnel with subepithelial connective tissue graft (SCTG). (A) Gingival recession defect <2 mm in depth at the maxillary right central incisor. (B) Appearance after tunnel preparation between the two lateral incisors. (C) Coronal advancement of the tunnel. (D) After mechanical debridement with ultrasonic and hand instruments, chemical root surface modification was accomplished with tetracycline hydrochloride (50 mg/mL). (E) Close-up view of tunnel preparation prior to SCTG insertion. (F) Subepithelial connective tissue graft harvest site. (G) The SCTG was trimmed to the dimensions of the recipient site. (H) The SCTG and tunnel flap were stabilized using interrupted sling sutures (4-0 dense polytetrafluoroethylene). (I) Complete root coverage was noted 9 months following the procedure.

recession defects in the mandibular anterior (Figure 3). Although controlled clinical research is lacking, multiple authors have successfully adapted tunneling techniques to root coverage and gingival augmentation in this anatomic region.[26,28,29]

### 2.2. Techniques incorporating external incisions

The methods described by Allen[5] and Zabalegui *et al.*[6] involved tunnel flap preparation through the gingival sulcus only. However, subsequent authors have suggested the addition of external incisions to facilitate recipient site preparation. In 2011, Dr. Homayoun Zadeh[10] introduced the vestibular incision subperiosteal tunnel access (VISTA) flap. This procedure began with thorough scaling and root planing, odontoplasty to reduce cervical prominences and bring the root within the alveolar housing, and root conditioning with 24% buffered EDTA gel. Four substantive distinctions between the VISTA approach and earlier tunnel flaps were the placement of vestibular access incisions to begin the tunnel preparation, elevation of a subperiosteal tunnel rather than utilizing a partial-thickness design, implantation of an absorbable porcine collagen membrane saturated in 0.3 mg/mL recombinant human platelet-derived growth factor-BB (rhPDGF-BB) rather than an SCTG, and use of bonded 6-0 polypropylene sutures to advance the facial flap margins coronally. However, a VISTA flap design can be applied when ADM or SCTG is implanted, and various suturing techniques can be utilized (Figure 4). A systematic review and meta-analysis found that a VISTA flap design with an ADM or SCTG yielded superior root coverage outcomes compared with tunnel flaps.[30]

Dr. Chao[11] introduced the pinhole surgical technique in 2012. In this technique, a small incision, 2–3 mm in length, was placed near the depth of the vestibule adjacent to the recipient site. A specialized transmucosal papilla elevator was inserted into the vestibular incision and used to elevate a full-thickness flap, which was extended laterally to include a minimum of four papillae. The flap was maintained in a coronal position by implanting two to four 2 × 12-mm absorbable porcine collagen membranes. No sutures, surgical dressings, or adhesives were applied to maintain the coronal position of the flap, and the vestibular access incisions were permitted to heal without suturing. In a long-term case series with a mean follow-up of 14.5 years, the pinhole technique demonstrated a CRC frequency of 78% and a mean root coverage of 94%.[31] Furthermore, in a split-mouth randomized clinical trial comparing clinical and patient-centered outcomes of the pinhole technique to those attained with CAF + SCTG, no significant difference between the two treatments was found.[32]

Tunnel flap preparation can be complex in the mandibular anterior region at sites presenting minimal recession depth and thin gingiva. In such situations, manipulating the flap with tunneling instruments can cause substantial trauma to the delicate marginal gingiva. Thus, in 2020, Dr. Allen[12] presented the papilla access

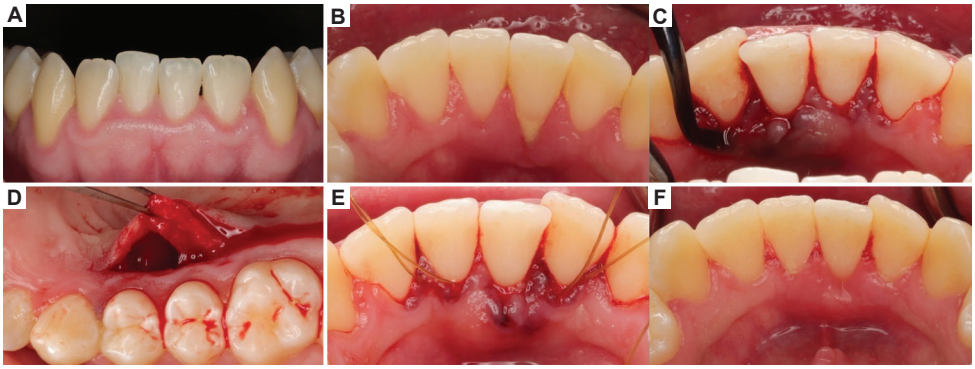


**Figure 3.** Tunnel flap with subepithelial connective tissue graft (SCTG) for treatment of lingual recession defects at mandibular incisor sites. (A) Baseline appearance of mandibular anterior teeth, facial view. (B) Baseline appearance of mandibular anterior teeth, lingual view. The mandibular right central incisor demonstrated a recession defect 3 mm in depth, a veneer of calculus, marginal erythema and edema, and bleeding upon periodontal probing. The apicocoronal dimension of the attached gingiva at this site was <1 mm. The remainder of the mandibular incisors exhibited lingual recession defects of <1 mm. (C) Preparation of the subperiosteal tunnel. (D) SCTG harvest. (E) Use of two 4-0 chromic gut sutures to position the SCTG within the tunnel. A subpapillary continuous sling suture (5-0 polypropylene) was used to stabilize the graft and tunnel flap (not shown). (F) Appearance of the site at post-operative week 6.

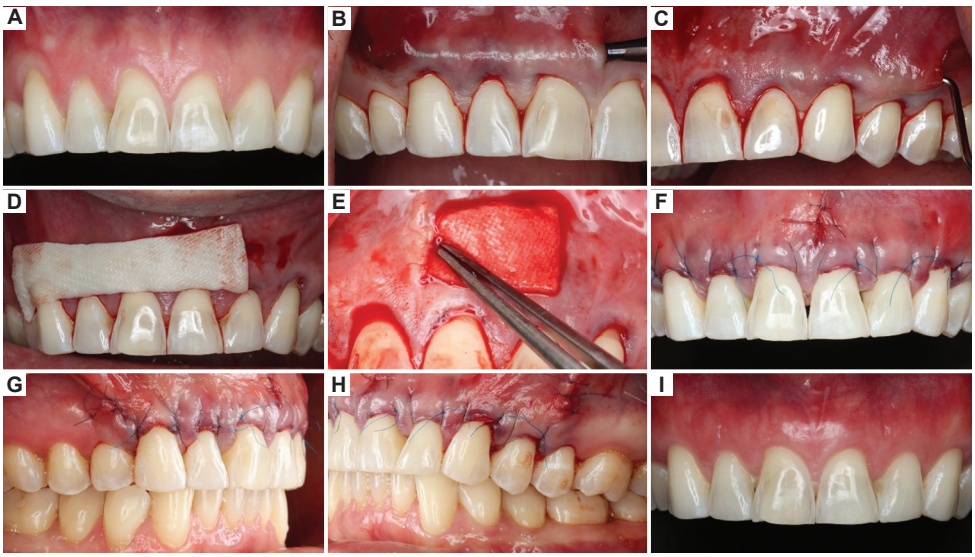


**Figure 4.** Vestibular incision subperiosteal tunnel access (VISTA). (A) Baseline clinical appearance of gingival recession defects at maxillary anterior teeth. (B) Periosteal elevator inserted through a small midline vestibular incision, demonstrating coronal advancement of the tunnel flap. (C) Tunneling instrument inserted in a vestibular incision distal to the maxillary left first premolar. (D) Acellular dermal matrix (ADM) overlying the vestibular incision. (E) The ADM was inserted through the midline vestibular incision and positioned at the cementoenamel junctions of the maxillary anterior teeth. (F) Closure using a subpapillary continuous sling suture (7-0 polyglycolic acid). Simple interrupted sutures were used to close vestibular incisions. (G) Closure viewed from the right side. (H) Closure viewed from the left side. (I) Clinical appearance eight weeks following the procedure.

tunnel (PAT) as an alternative to vestibular access incisions. The technique involved sharp dissection of one or more facial papillae to permit access for tunnel flap preparation (Figure 5). When treating all mandibular anterior teeth, the author recommended partial-thickness dissection and reflection of the papillae between the lateral incisors and canines bilaterally. The adjacent tunneled papillae were detached from the interdental alveolar bone using a curette. After positioning of the selected graft/biomaterial—SCTG, ADM, or xenograft—the PAT was coronally positioned to the CEJs and stabilized using interrupted or continuous sling suturing.

In 2023, Marques *et al.*[13] published the mixed-thickness tunnel access (MiTT) technique. The MiTT involved vertical access incisions terminating approximately 2 mm apical to the MGJ and split-thickness separation of the alveolar mucosa from the underlying muscles using specialized tunneling instruments. From the MGJ, a full-thickness tunnel flap was reflected to the gingival margin,

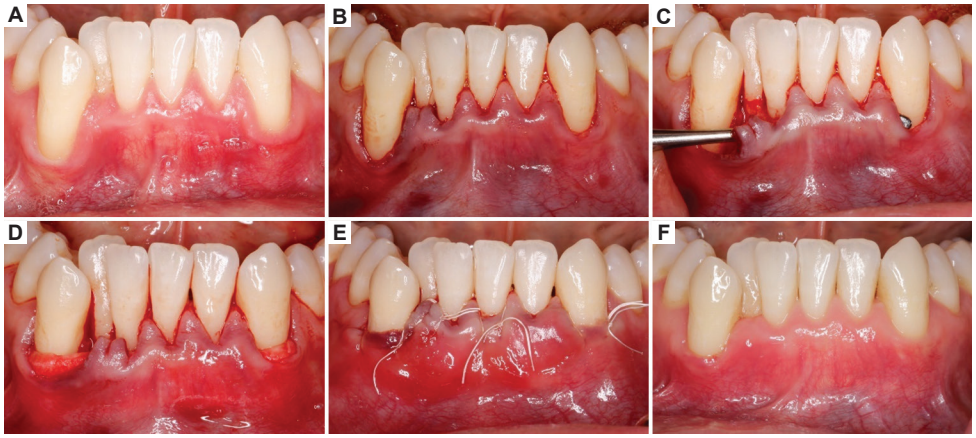


**Figure 5.** Modified papilla access tunnel (PAT). (A) Baseline appearance of mandibular anterior teeth. Crowding, supereruption of the incisors, and malposition of multiple teeth were noted. (B) Initial incisions and tunneling. A PAT typically involves reflection of one or more papillae. Due to the crowding in this case, the technique was modified. The small segment of facial gingiva overlying the right lateral incisor was reflected. (C) A subperiosteal tunnel was accomplished through the gingival sulcus and the modified PAT. A scalpel was used to release the tunnel flap apically. (D) An acellular dermal matrix (ADM) was implanted in the tunnel from canine to canine. (E) The ADM and coronally advanced tunnel were stabilized with interrupted sling sutures. (F) Appearance of the outcome at post-operative week 6.

with or without the use of intrasulcular incisions. The base of each papilla was reflected. However, the tip remained intact. Within the tunnel, the authors recommended positioning the coronal margin of an SCTG or a de-epithelialized gingival graft 1 mm coronal to the CEJ.

## 3. Tunnel flaps in ARA

Various tunneling techniques have been applied to ARA in an attempt to reduce the risk of the most common complication associated with the procedure—wound dehiscence and exposure of implanted biomaterials.[33] In 2007, Kfir *et al.*[14] presented a series of 11 cases demonstrating the use of tunnel flaps for guided bone regeneration (GBR). The technique involved placement of a vertical vestibular incision through which a full-thickness mucoperiosteal pouch was established with the aid of a silicone catheter and an inflation syringe. An absorbable membrane was inserted through the vestibular incision, and a mixture of autologous fibrin and beta-tricalcium phosphate was implanted between the membrane and the alveolar bone.

Nevins *et al.*[15] published a series of 12 cases involving a tunnel-based minimally invasive ARA technique in 2009. In this method, subperiosteal pouches were established through vestibular incisions, and mixtures of rhPDGF-BB and anorganic bovine bone mineral (ABBM), ABBM with mineralized collagen bone substitute (MCBS), or freeze-dried bone allograft were applied without the use of membranes. In two patients who had received ABBM + MCBS, implant placement was not possible due to the quality or volume of hard tissue. Histologic analyses revealed combinations of residual biomaterial particles, newly formed bone, and fibrous connective tissue. Multiple specimens from sites that had received MCBS revealed biomaterial particles encapsulated in connective tissue.

In 2017, Dr. Lee[16] introduced the subperiosteal minimally invasive esthetic ridge augmentation technique, which involved establishing a subperiosteal tunnel and implanting ABBM hydrated in rhPDGF-BB, without the use of a barrier membrane. One or more vestibular incisions were placed distant to the deficient alveolar ridge, through which a tunnel flap was prepared. The bone biomaterial and growth factor mixture was applied through the vestibular incisions. Most patients reported little or no discomfort and/or swelling. Histologic analysis revealed new bone in direct apposition with ABBM particles and dense connective tissue. This approach was applied at 60 sites in a series of 21 patients; the mean increase in horizontal ridge width was approximately 5 mm.

Johnson and Baron[17] utilized tunnel access for GBR with a nonabsorbable membrane in the maxillary lateral incisor area (Figures 6 and 7). The authors made two vestibular incisions adjacent to the alveolar ridge deficiency, reflected a full-thickness mucoperiosteal tunnel flap extending palatally over the alveolar crest, and inserted a dense polytetrafluoroethylene membrane, which was tailored to the dimensions of the site. A particulate freeze-dried bone allograft was packed between the alveolar bone and the barrier membrane. The gingival attachment at the adjacent teeth was released, permitting coronal advancement of the tunnel flap. The patient reported minimal discomfort limited to the first 2 post-operative days, and the procedure resulted in a favorable alveolar ridge volume for implant placement.

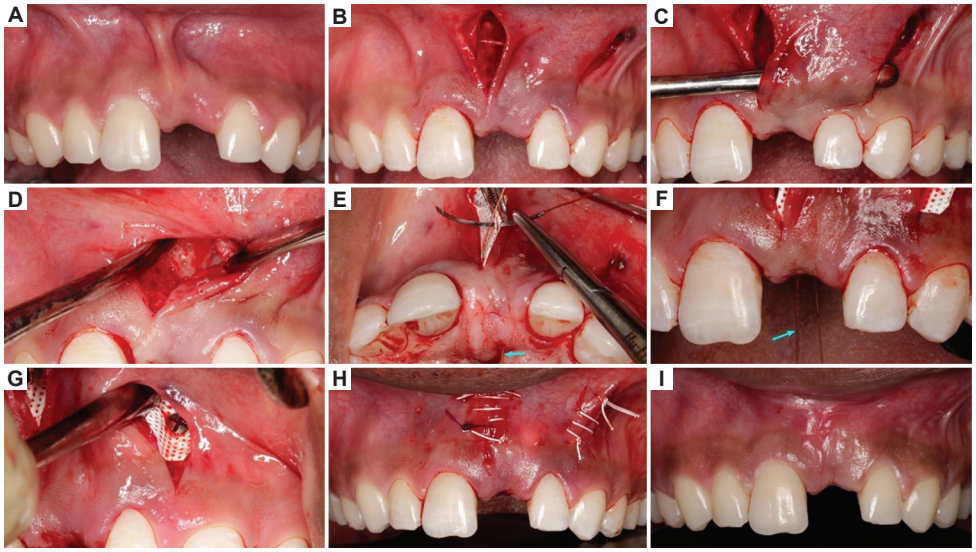


**Figure 6.** Tunnel access for guided bone regeneration (GBR). (A) Baseline appearance of maxillary anterior teeth. At the left central incisor position, a substantial undercut in the alveolar bone could be palpated and appreciated visually. A cone-beam computed tomography scan confirmed inadequate bone volume for implant placement. (B) Vertical vestibular incisions were placed in the midline frenum and between the left canine and lateral incisor. The incisions extended from the depth of the vestibule 2–3 mm into the attached gingiva. A subperiosteal tunnel was established between the two vestibular incisions. The full-thickness reflection extended palatally over the alveolar crest. A periosteal releasing incision was placed at the apical aspect of the tunnel. (D) Intramarrow penetrations were established through the vertical incisions. (E) A dense polytetrafluoroethylene (PTFE) membrane was trimmed to the dimensions of the site and inserted into the tunnel. A 4-0 chromic gut suture (arrow) needle was passed from the palatal aspect of the tunnel through the midline vertical incision. The suture engaged the portion of the membrane designed to extend over the alveolar crest. (F) The needle was then passed through the midline vertical incision, exiting the palatal mucosa adjacent to the original needle entry point (arrow). The membrane was then drawn into place by exerting tension on both ends of the suture. (G) After implanting autogenous bone shavings and a freeze-dried bone allograft, the membrane was stabilized with two fixation screws and an absorbable 5-0 polyglycolic acid mattress suture. (H) The vestibular incisions were closed with simple continuous 4-0 dense PTFE sutures. (I) Appearance of the site 6 months following GBR.

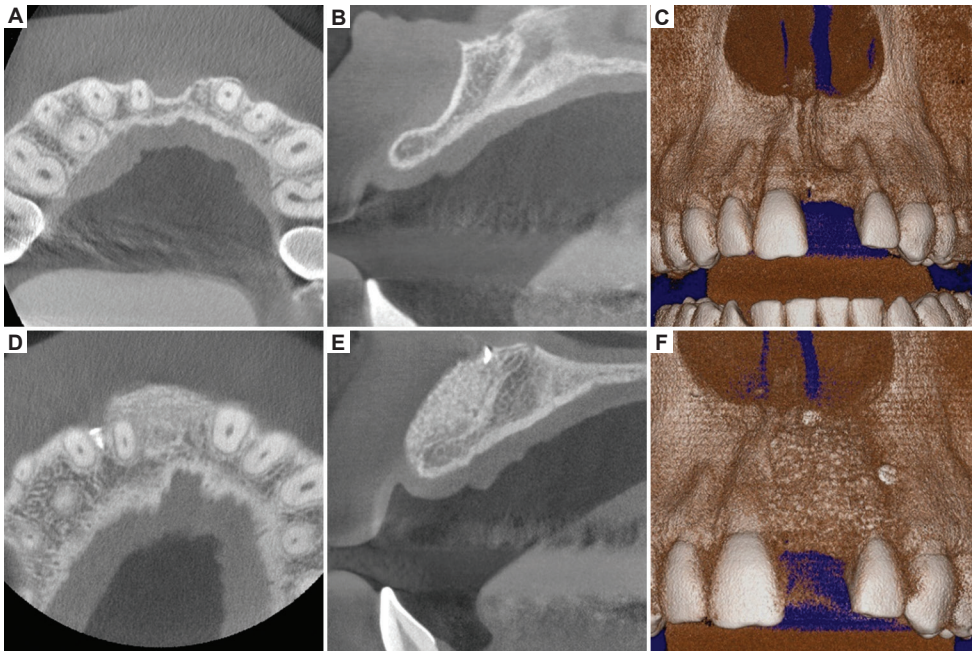


**Figure 7.** Comparison of baseline and follow-up cone-beam computed tomography volumes. (A) Baseline axial slice. (B) Baseline cross-sectional slice. (C) Baseline volume rendering. (D) Axial slice 6 months following the procedure. (D) Cross-sectional slice 6 months following the procedure. (E) Volume rendering 6 months following the procedure.

In 2020, Karmon *et al.*[18] introduced a tunneling technique for horizontal ARA using a subperiosteal bag. The technique involved folding, suturing, and perforating a collagen membrane containing a deproteinized bovine bone derivative. A vertical vestibular incision was placed adjacent to the alveolar ridge deficiency, through which a subperiosteal tunnel was reflected. The bag containing a particulate xenograft was then implanted in the tunnel with the perforated side facing the alveolar bone. Three patients received ARA using this technique. Each procedure resulted in sufficient alveolar ridge volume for implant placement, and all patients reported minimal discomfort.

## 4. Tunnel flaps in regenerative periodontal therapy

In regenerative periodontal surgery, a clear trajectory from conventional flap techniques toward minimally invasive methods has emerged over the last half-century. From the late 1960s to the late 1980s, reports were published confirming histologic periodontal regeneration—formation of new bone, cementum, and periodontal ligament—at intrabony periodontal defect sites treated with autogenous bone implants, bone derivatives, and guided tissue regeneration.[34-37] In subsequent years, skilled clinician–researchers carefully identified patient-, tooth-, defect-, procedure-, and operator-related factors relevant to the establishment of periodontal regeneration.[38] Wound closure, space maintenance, and clot stability were recognized as surgical prerequisites.[39,40] In the 1990s, variations of conventional mucoperiosteal flaps were developed to maximize wound closure for primary intention healing over barrier membranes and biomaterials implanted at IBD sites.[41,42]

Later, Dr. Cortellini and Tonetti[43,44] advocated for increasingly less invasive surgical methods, introducing first the MIST,[43] then the M-MIST.[44] Compared with conventional flap techniques, these procedures limited access to the root surface for debridement but emphasized wound closure and clot stability.[43,44] The M-MIST represented a refinement of the original technique to reduce patient morbidity further, minimize collapse of the interproximal gingiva, maximize space maintenance, and enhance wound/clot stability.[44] It involved reflection of only a buccal/facial papillary flap. The oral papilla remained intact, and the granulation tissue was sharply dissected from the lingual soft tissue and bone using a microblade and removed using a mini-curette. Favorable periodontal stability after 10 years of follow-up has been observed at IBDs treated with the M-MIST alone, M-MIST + EMD, and M-MIST + EMD + bone derivative.[3]

The M-MIST evolved further with the advent of the entire papilla preservation technique (EPPT).[19,45-47] The EPPT completely avoided reflection of any portion of the IBD-associated papilla.[19,45] Instead, a vertical incision was shifted to an adjoining tooth.[19] A small full-thickness flap was reflected between the vertical incision and the IBD, and the defect-associated papilla was approached in a "tunnel-like" fashion. Microsurgical scissors and mini-curettes were used to remove the interproximal granulation tissue.

Two additional tunneling techniques have been devised to access deep IBDs without incision of the defect-associated papilla. Moreno Rodríguez and Caffesse[20] developed the non-incised papilla surgical approach (NIPSA). In this procedure, one apical horizontal or oblique incision was made within the alveolar mucosa. Through this access, the granulation tissue was removed, the root surfaces were debrided, and biomaterials/EMD were implanted.[20,48] In a comparative analysis including NIPSA and MIST procedures, the two techniques produced similar clinical results.[20] However, NIPSA resulted in lower recession and superior soft-tissue preservation.[20,48] Meanwhile, Pohl and Buljan[21] introduced the VISTA technique for regenerative treatment of IBDs. The technique combined VISTA access with application of a bone allograft, EMD, and an SCTG. Favorable clinical outcomes and PROMs were observed.

## 5. New tunneling applications in periodontics

### 5.1. Circumferential tunneling in periodontal plastic surgery

Gingival recession caused by mechanical factors, such as tooth brushing, is typically restricted to the facial surfaces of teeth, whereas recession caused by periodontitis can occur in a circumferential pattern and may be irreversible.[49,50] Nevertheless, clinicians occasionally encounter teeth exhibiting both oral and facial gingival recession defects that are not attributable to periodontitis. Although the vertical height of the interproximal alveolar crest may be normal, dehiscence defects at oral and facial surfaces may be present, and the interproximal bone may be thin and delicate. In such situations, circumferential tunneling is a potential treatment option for achieving root coverage at both facial and palatal/lingual surfaces (Figure 8). Controlled clinical research is needed to establish the predictability of this method.

### 5.2. Multi-surface tunnel preparation in the treatment of periodontitis

Incorporating gingival augmentation into regenerative periodontal therapy at periodontal defect sites that demonstrate soft tissue deficiency has been recommended.[51] Dr. Zucchelli *et al.*[52,53] proposed the use of the connective tissue graft wall technique to replace a deficient or missing

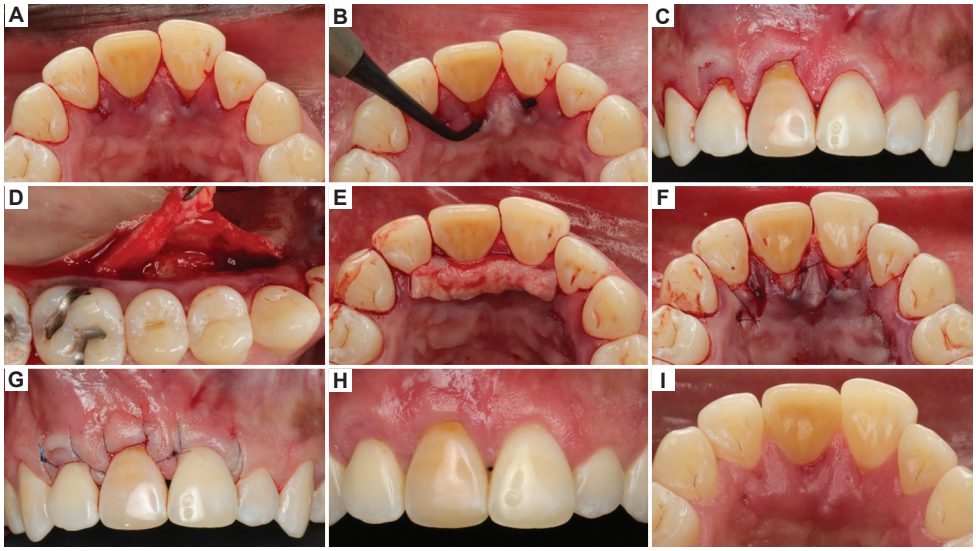


**Figure 8.** Circumferential tunneling for root coverage. (A) Subperiosteal tunnel established at the maxillary central incisor palatal recession sites. (B) Tunneling instrument demonstrating a patent tunnel between the central incisors. (C) A facial tunnel was established to address gingival recession at the right lateral and central incisors. (D) Subepithelial connective tissue graft (SCTG) harvested. (E) The SCTG was trimmed to the dimensions of the palatal tunnel. (F) The SCTG was stabilized with interrupted 7-0 polyglycolic acid sling sutures. (G) A subpapillary continuous sling suture was used to coronally advance the facial tunnel without the addition of a second SCTG. (H) Facial view of maxillary incisors 3 months following the procedure. (I) Palatal view of maxillary incisors 3 months following the procedure.

IBD wall, enhance space maintenance for periodontal regeneration, and minimize facial and interproximal recession. The procedure has been shown to result in significant reduction in probing depth and gain in clinical attachment, while also producing root coverage and improved papilla fill. Rather than reflecting a conventional flap, an SCTG wall can be accomplished through multi-surface tunneling. Figure 9 demonstrates the establishment of an SCTG wall through a lingual tunnel, without the need for conventional flap reflection. Extending the tunnel interproximally and facially permitted access for complete debridement of the IBD, proper instrumentation of root surfaces, and coronal advancement of the interproximal gingiva, with stability achieved using a bone allograft.

## 6. Discussion

The purpose of this report is to review the evolution of tunnel-based surgical techniques in periodontics and to suggest new tunneling applications within the field. Multiple minimally invasive techniques have become increasingly utilized in periodontics due to documented long-term stability of results and high patient acceptance.[3,23,26,54,55] In periodontal plastic surgery, clinicians have long acknowledged that tunnel-based root coverage procedures often lead to superior patient-oriented outcomes, characterized by reduced discomfort and swelling, faster return to daily activities, and favorable early esthetic results.[10-13,28-32,54,55]

Nevertheless, mixed results appear in the literature when comparing clinical outcomes of tunnel-based root coverage procedures versus those attained through conventional flap techniques.[23,56-59] Stabilizing the flap margin in a coronal position beyond the CEJ is a procedure-related factor that has been associated with CRC.[60] Accomplishing this degree of advancement may be more difficult when a tunnel flap is utilized. For example, the described post-surgical position of the PAT flap margin was the CEJ.[12] Likewise, the MiTT involved positioning the graft—not the flap—1 mm coronal to the CEJ.[13] Zabalegui *et al.*[6] did not attempt to coronally advance the tunnel flap, intentionally leaving portions of the implanted SCTG exposed during healing. However, the magnitude of flap advancement after suturing is known to influence the treatment outcome—the more coronal the flap margin at the completion of surgery, the greater the likelihood of CRC.[60]

Tunneling techniques that include external incisions may offer clinically relevant advantages. Both the PAT and VISTA techniques enhance access for tunnel flap preparation, graft or biomaterial placement, and flap release for coronal advancement. These techniques may also reduce surgical trauma to the marginal gingiva and decrease operating time. Indeed, in a systematic review and meta-analysis, outcomes following VISTA + ADM or SCTG surpassed results obtained through tunneling without external incisions.[30]

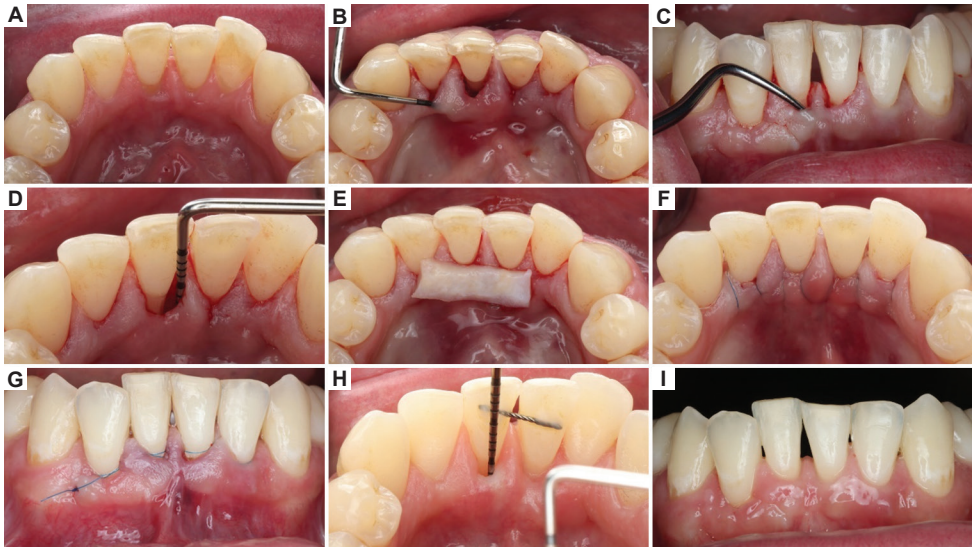


**Figure 9.** Multi-surface tunnel preparation for the connective tissue graft wall technique. (A) Lingual recession at mandibular central incisors. The left central incisor exhibited 9-mm probing depths at the mesiolingual and direct lingual aspects. (B) Intrasulcular incisions were made with ophthalmic microblades, and a lingual subperiosteal tunnel was established to facilitate debridement of the defect, thorough root planing, and positioning of a de-epithelialized gingival graft (DGG). (C) The tunnel preparation extended to the interproximal and facial surfaces to permit complete instrumentation of the affected root surfaces and coronal advancement of the midline papilla. (D) Appearance of the root surface after debridement. (E) The DGG was harvested from the palate, de-epithelialized extraorally, and tailored to the dimensions of the site. (F) A demineralized freeze-dried bone allograft (DFDBA) was applied through the facial tunnel access against the lingual connective tissue graft wall. The DFDBA helped maintain the midline papilla in a coronal position. The DGG and lingual tunnel flap were stabilized using a subpapillary continuous sling suture (7-0 polyglycolic acid). (G) Immediate post-operative appearance, facial view. The two mandibular central incisors were splinted, and occlusion was adjusted to avoid excessive force on the affected tooth. (H) 3 months following the procedure, all probing depths were ≤3 mm. (I) Facial view of mandibular anterior teeth 3 months following the procedure.

Evidence supporting tunnel-based ARA procedures is limited to case reports/series.[14-18] However, positive results documented in initial reports suggest that controlled clinical studies are warranted. The most common post-operative complication of GBR is wound dehiscence and membrane exposure at the incision line.[33] It is possible that accomplishing GBR without the need for a horizontal incision at the alveolar crest may reduce the occurrence of wound dehiscence. Achieving ARA using a subperiosteal tunnel may also simplify closure, reduce the procedure duration, and limit patient morbidity.

Tunnel applications in regenerative periodontal surgery represent iterations of prior minimally invasive procedures that have been validated through long-term clinical investigation.[3,41,42] All these procedures balance two critical concerns—clot stability and access to the root surface for addressing the etiology. Additional controlled clinical research and comparative analyses are needed to define the relative efficacy of emerging techniques. In principle, however, the conditions for periodontal regeneration that clinicians must establish intraoperatively have not changed.[37,38] Tunneling is merely a means of establishing these conditions.

Integrating tunnel-based techniques into graduate dental education presents a dilemma for educators. Despite generally consistent reports that tunneling can favorably influence PROMs, available evidence does not imply that tunnel procedures should completely replace more established methods. Thus, students must become proficient in using both conventional and tunnel flap designs. However, tunnel-based procedures may be more technique-sensitive and thus challenging for inexperienced operators. A reasonable approach may be to start new residents using conventional flaps and introduce tunneling after the students have gained additional surgical experience and confidence.

Multiple tunnel-based procedures have utilized specialized surgical instruments. For example, in the modified microsurgical tunnel technique, Zuhr *et al.*[7] utilized special tunneling knives during tunnel preparation. Meanwhile, Chao[11] and Chao *et al.*[31] utilized a specialized transmucosal papilla elevator to accomplish the pinhole procedure for root coverage. Both the MiTT and the MCAT relied upon specifically designed tunneling instruments.[9,13,24] Certainly, it is possible to establish a tunnel preparation without the benefit of specialized instrumentation. Nevertheless, such instruments may augment the operator's ability to achieve adequate flap reflection and release without causing undue trauma to the delicate marginal gingiva.

## 7. Conclusion

Tunneling procedures represent a subset of the minimally invasive surgeries that have emerged in contemporary periodontics. Virtually all tunneling procedures aim to limit patient morbidity, enhance wound stability, and avoid exposure of implanted membranes, grafts, and biomaterials. The procedures often represent refinements of techniques that involve reflection of crestal or papillary gingival tissue. Tunnel flaps for root coverage derive from one of the simplest procedures used to treat isolated gingival recession defects—the Raetzke pouch. Subsequent authors have expanded tunneling to sites exhibiting multiple adjacent recession defects and enhanced the procedures using external incisions, specialized instruments, distinct surgical protocols for addressing the interproximal gingiva, advanced suturing techniques, and implantation of various grafts and biologics. In periodontal regeneration, tunneling techniques represent the natural extension of highly successful and well-validated M-MIST. Tunnel-based ARA procedures are intriguing; however, supporting evidence is limited to case reports. This narrative review recounts the evolution of tunneling within periodontics and suggests opportunities for further derivations of the technique. Multi-surface and circumferential tunneling may be applicable in specific clinical circumstances.

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## Conflict of interest

The authors declare that they have no competing interests.

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## Ethics approval and consent to participate

Each patient completed an informed consent process involving verbal and written components. The consent process included completion of a standardized photographic release form for training, education, research, and publication. All consent forms are maintained in the appropriate patient electronic health records.

## Consent for publication

Patients consented to the publication of their data.

## Availability of data

Not applicable.

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PERSPECTIVE ARTICLE

# Perspectives: Recent advances in community-based interventions for cardiovascular disease disparities in the United States

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## Abstract

Despite decades of progress in prevention and management, cardiovascular disease (CVD) remains the leading cause of mortality in the United States (US) and globally, with persistent and even widening disparities in outcomes across racial, ethnic, geographic, and socioeconomic groups. As a complement to clinical care, community-based interventions have emerged as vital tools in promoting cardiovascular health, particularly in underserved populations. This perspective focuses on recent advances in US-based community interventions for CVD, while acknowledging global relevance, and explores the historical evolution, current landscape, and future directions of community-driven CVD prevention strategies. We highlight foundational models such as the Healthy Heart Community Prevention Project in New Orleans, Louisiana, and examine recent major studies and innovations in the field from the US and across the world. Community-rooted programs demonstrate growing potential to address upstream determinants of cardiovascular health. Their continued success, however, will depend on sustained investment, robust evaluation frameworks, and alignment with clinical care pathways and health policy infrastructure.

***Keywords:*** Community interventions; Cardiovascular disorders; Hypertension; Disparities

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## 1. Introduction

Cardiovascular disease (CVD), including coronary artery disease, myocardial infarction, heart failure, cerebrovascular disease, and other related disorders, is the primary cause of morbidity and mortality worldwide, including the United States (US). Despite significant advancements in prevention and treatment, recent data suggest that CVD rates continue to rise.[1,2] While age-adjusted mortality rates may have slightly declined, the total number of CVD-related deaths increased by over 10,000 in the US from 2021 to 2022, indicating the ongoing need to identify and implement more effective strategies for CVD prevention and control.[1] Traditional clinical care, including visits to physicians, nurse practitioners, and other clinicians, remains essential to control cardiometabolic risk factors such

as obesity, diabetes, hypertension, hyperlipidemia, and smoking; however, growing evidence suggests that community-based interventions, beyond simple screening, may be a strong pathway to optimize the control of these risk factors.[3-6] In the US, CVD-related healthcare costs including direct costs, related to disease management and indirect costs in the forms of lost productivity and early mortality, accounted for approximately US$ 400 billion dollars in 2019.[7] Based on 2022 US dollar value, annual inflation-adjusted CVD spending is projected to increase to US$1.8 trillion by 2050.[8] These alarming forecasts highlight the urgent need to prioritize and expand efforts in CVD prevention, focusing on community-based interventions, which have the potential to offer cost-effective measures that substantially reduce healthcare costs imposed by CVD. In this perspective piece, we explore recent advancements in community-based cardiovascular interventions, emphasizing recent US-based advancements, while drawing connections to select global initiatives where relevant. We examine strategies such as digital health programs, community-led behavioral interventions, and policy-driven approaches, with an emphasis on their impact on health equity. While this perspective focuses on marginalized and underserved communities, it is important to recognize that community-based interventions to promote a healthier lifestyle and minimize CVD risk factors can, and should, be implemented in all populations.

## 2. Methodology

For this perspective, we conducted a literature review using PubMed, focusing on studies published in English between 2023 and 2025, to capture recent advancements in community-based cardiovascular interventions. Our search strategy combined MeSH terms and title or abstract keywords related to CVDs, community health services, and implementation science. We prioritized original research, including randomized controlled trials (RCTs), community trials, and implementation studies, while excluding meta-analyses and systematic reviews. Although the systematic search included only studies from 2023 to 2025, we incorporated relevant older studies where appropriate to provide historical context and strengthen key discussions. The review encompassed lifestyle, digital health, policy-based, and culturally tailored interventions aimed at improving cardiovascular outcomes, particularly in underserved populations.

## 3. Brief history of the evolution of community-based CVD interventions

Community-based cardiovascular interventions have evolved significantly over the past several decades, shifting from broad public health campaigns to more targeted, culturally tailored approaches. Early large-scale initiatives, such as the Framingham Heart Study and the National Health and Nutrition Examination Survey, laid the groundwork for understanding cardiovascular risk factors at the population level.[9-11] However, these studies primarily informed clinical guidelines rather than directly engaging communities. The realization that traditional healthcare settings alone were insufficient in preventing CVD and addressing disparities led to the emergence of community-driven interventions, particularly for high-risk populations.

One of the earliest and most influential models was the Church/Community Health Awareness and Monitoring Program in Baltimore, Maryland, during the late 1970s.[12-14] This initiative mobilized church volunteers from roughly 100 local churches to conduct blood pressure screenings and referrals in predominantly Black communities. This approach laid the foundation for later barbershop-based hypertension programs, where trusted community figures facilitated engagement in cardiovascular care. In the 1990s, the Healthy Heart Community Prevention Project (HHCPP), led by New Orleans locals Daphne Ferdinand, PhD RN, and Keith Ferdinand, MD, in New Orleans, Louisiana, expanded on this model, integrating faith-based health messaging and barbershop and hair salon hypertension screening programs to improve cardiovascular awareness in African American populations.[15,16]

The early 2000s saw the rise of policy-driven cardiovascular interventions, including the Million Hearts initiative, a national program launched in 2012 by the Centers for Disease Control and Prevention and the Centers for Medicare & Medicaid Services to prevent one million heart attacks and strokes within 5 years.[17] The initiative focuses on system-wide improvements in cardiovascular care and collaborates with community organizations, pharmacies, local health departments, faith-based groups, and employers to promote hypertension control, smoking cessation, and heart-healthy behaviors beyond traditional clinical settings. In addition, studies such as the Los Angeles Black Barbershop Blood Pressure Study demonstrated the power of pharmacist-barber collaborations, achieving significant blood pressure reductions through community-based medication management.[18]

Internationally, the North Karelia Project in Finland, launched in 1972, remains one of the most successful large-scale community interventions.[19] By targeting dietary changes, smoking cessation, and blood pressure control through community and government collaboration, North Karelia achieved remarkable reductions in CVD mortality

over several decades. These historical efforts have paved the way for modern interventions which will be discussed in the following sections.

## 4. Health equity and community-based cardiovascular interventions

Disparities in cardiovascular health across racial, ethnic, and socioeconomic lines remain a major public health crisis in America. Black, non-black Hispanic, indigenous, and rural communities in America experience significant health disparities and are at a notably higher risk for CVD. Compared to White Americans, Black Americans are nearly twice as likely to have major CVD risk factors such as obesity, type 2 diabetes, and hypertension, and to experience major CVD including ischemic heart disease, heart failure, and stroke.[20] In addition, non-Hispanic Black individuals are 33% more likely to die of CVD than non-Hispanic white individuals.[21] Recent data from 43,000 persons also confirmed that Black women and men have the highest long-term cardiovascular mortality of any racial or ethnic group, even after adjusting for atherosclerotic CVD risk scores and coronary artery calcium.[22] These disparities can be attributed to social determinants of health, including healthcare access, low socioeconomic status, food deserts, racism-related stress, and lack of trust in the healthcare system.[23]

For Hispanic communities, language barriers provide their own complications. Furthermore, a lack of Hispanic clinicians, lower socioeconomic markers among Hispanic immigrant communities, and food deserts caused by geographic disparities add to the challenges to reduce CVD burden in this population.[24] Large meta-analyses have found that Hispanic Americans have similar levels of minor CVD as White Americans but much higher incidence of type 2 diabetes.[20] However, a unique challenge in studying Hispanic populations in America is their incredible diversity, both genetically and socio-culturally. The term "Hispanic" is an umbrella classification that includes individuals of varying national origins, racial backgrounds, migration histories, and socioeconomic statuses. This heterogeneity poses methodological challenges in cardiovascular research, as findings from one subgroup (*e.g.*, Mexican Americans) may not be applicable to another (*e.g.*, Puerto Ricans, Cubans, or Central Americans). Consequently, broad generalizations may undermine the credibility of study findings. As a result, some studies may find substantial CVD disparities, while others find none.[24,25]

Indigenous US communities also suffer from higher rates of CVD than their non-Hispanic White counterparts. Indigenous men and women have, respectively, 30% and 70% higher mortality rates from CVD compared to the general population. The unique culture, history, and legal status of indigenous communities provide unique challenges and opportunities for community-based health interventions.[26]

In addition, rural communities face their own unique challenges in managing cardiovascular risk. Lack of both specialized and primary care physicians and other clinicians, lack of medical supplies, long travel distances to hospitals, and high incidence of food deserts contribute to a stark urban/rural divide in markers of cardiovascular health. Although poverty level is a strong moderating variable, rural areas have higher CVD death rates than their urban counterparts across the board. These disparities are the most pronounced in the southern US, where rural populations experience over double the rate of CVD mortality.[27] Community-based health interventions are critical pieces in boosting healthcare within underserved communities and bridging gaps in health equity. Crucially, these interventions must consider the differences in specific patient populations to be effective. In African–American communities, involving non-medical community members such as hairdressers and pastors has been shown to increase medication adherence as well as primary and secondary health outcomes.[16] Medication adherence, notably reduced in Black communities, is a major risk factor that can be attributed to lower education levels, lack of social support and culturally competent care, and high costs. Many of these factors can be directly addressed with a community-based intervention involving other persons in addition to physicians. A notable successful example of combining health professionals with community leaders is the Los Angeles Barbershop Blood Pressure Study, where pharmacist-led interventions in Black-owned barber shops drastically reduced blood pressure.[18] On the other hand, one shortcoming of not involving physicians in community-based initiatives is a lack of integration between different types of healthcare workers and actual clinical care.[28]

Recent innovations include the use of geographic information system mapping to determine the optimal locations of so-called "trusted spaces" – community centers such as barber shops and nail salons where staff can provide evidence-based care to maximize the positive impacts.[29] In Hispanic communities, programs targeting language and cultural barriers have been particularly effective. Thirteen weeks of behavioral classes on healthy habits taught in Spanish to patients with type 2 diabetes decreased hemoglobin A1c (HbA1c) from an average of 7 – 6.3% after 1 year.[30] This is especially significant considering diabetes is the main cardiovascular disparity that is consistently

identified in Hispanic populations. Similar positive results are seen in North American indigenous populations. A meta-analysis of 1986 studies on CVD in indigenous communities suggested that adapting a model of heart health that incorporates traditional indigenous notions of heart health and integrating community members into care vastly improved health outcomes. Patient education programs incorporating modern medicine with traditional indigenous storytelling and ceremony were especially valuable in improving outcomes or older indigenous persons.[26] Trials conducted in rural settings, both in the US and internationally, have also demonstrated significant benefits. A recent intervention conducted in rural China, where patients with uncontrolled hypertension were assigned to a non-physician community-based provider, was associated with a reduction in systolic blood pressure by 23 mmHg compared with usual care.[31] Studies in rural Kentucky examining the effect of community health workers on patients with type 2 diabetes found that the workers markedly improved patient confidence and self-worth but lagged in improving heart-healthy behavior or HbA1c levels. This underscores the need to develop new strategies that are molded to specific populations.[28] Community health interventions could define a new standard of care for CVD prevention, but it is imperative that these programs are adjusted based on individual communities and that their planning involves the voices of those communities. Much more work needs to be done to establish how community interventions can be employed in different geographic and demographic areas. However, navigating a dynamic political climate to ensure a consistent stream of funding is a critical factor for all researchers.

## 5. Key innovations in community-based cardiovascular interventions

The HHCPP emerged during the 1990s as a pioneering community-based initiative to reduce cardiovascular risk in African–American populations.[15,16] It focused on building strong partnerships within the backbone of the community including with barbershops, beauty salons, churches, and healthcare professionals to promote healthy lifestyle changes in a culturally relevant fashion that resonated with the community. Major outreach activities included blood pressure screenings during barbershop visits, health messages delivered during church worship services, clinical symposia for local healthcare professionals aimed at improving hypertension and lipid guideline adherence, and efforts to reduce vaccination hesitancy. Today, HHCPP remains active through various community outreach programs such as health fairs, healthy cooking classes, health education and faith-based programs, as well as expanded academic collaborations to generate scientific evidence from its community initiatives.

One of these relevant local initiatives, the Church-based Health Intervention to Eliminate Racial Inequalities in Cardiovascular Health (CHERISH) Study, uses the potential of faith-based community interventions in addressing cardiovascular disparities.[32] Funded by the National Institutes of Health (NIH), CHERISH is a 7-year RCT partnering with over 40 predominantly African–American churches across New Orleans. The intervention trains community health workers to deliver cardiovascular prevention strategies aligned with the 2019 American College of Cardiology/American Heart Association guidelines.[33] By embedding health promotion into trusted church environments, CHERISH aims to reduce disparities in blood pressure, cholesterol, and overall cardiovascular risk through education, coaching, and sustained behavioral support.

Another HHCPP effort, the Text My BP Meds NOLA study, was an innovative digital health initiative designed to improve hypertension control in Black adults residing in New Orleans.[34] The program utilized simple cell phone text message-based technology to deliver regular messages to participants regarding medication adherence and blood pressure self-monitoring. The study showed significant improvements in medication adherence and blood pressure.

In a more recent academic partnership with Tulane University, and in response to the increasing rates of obesity and use of glucagon like peptide-1 agonists (GLP-1a) for cardiometabolic disease management, HCCP is working on a pilot community-based intervention that will integrate culinary classes, cardiovascular health education, and personalized digital meal-planning tools, to enhance the effects of GLP-1a, preserve muscle mass, and promote sustainable lifestyle changes. Currently submitted for NIH funding through the Louisiana Clinical and Translational Science Center, the program seeks to equip participants with skills and resources that persist even when access to GLP-1a medications is lost due to any reason including insurance coverage issues which frequently occurs.[35]

Recent evidence from large-scale trials further supports the strategies embedded within programs such as the HHCPP. A 2024 meta-analysis of digital health interventions for management of hypertension in the US populations experiencing health disparities showed that most of the 28 included studies examined multicomponent digital health interventions incorporating digital health (remote blood pressure monitoring in this case), community health workers or nurses, and/or cultural modifications, components critical to community-based

interventions and incorporated into the studies by the HHCPP.[36] This analysis also revealed that community interventions led to significant reductions in blood pressure compared with the standard treatment groups. While not a community-based intervention per se, the 2024 RICH LIFE trial demonstrated that multilevel, equity-focused strategies within primary care settings such as incorporating social needs screening and team-based management can significantly improve blood pressure control in high-risk populations, particularly in rural areas and among patients with coronary heart disease.[37] The SAHELI, an RCT performed in Chicago, Illinois, among the US South Asian adults, investigated a 16-week culturally adapted group lifestyle intervention versus written health education materials to reduce CVD risk factors including blood pressure, cholesterol, and HbA1c. Although the study did not meet its primary outcome of improving the CVD risk factors, the intervention was at least associated with minor improvements in self-reported health behaviors, which can be a stepping stone in the long-term management.[38]

In addition to efforts within the US, several recent community-based RCTs conducted globally have yielded overwhelmingly positive results. A large 2023 RCT studying a geographically diverse population of 33,995 participants in China showed that participants receiving a blood pressure intervention led by non-physician community healthcare professionals had significantly lower rates of major cardiovascular events including a composite of myocardial infarction, stroke, heart failure requiring hospitalization, and CVD-related death after 36 months compared with the standard care group (hazard ratio [HR]: 0.67, 95% confidence interval: 0.61 – 0.73). They also found a significant reduction in blood pressure in the intervention group.[30] Another Chinese RCT evaluated a digital health behavioral intervention for older adults with hypertension.[39] The intervention utilized a social media-based messaging platform and health education, incorporating personalized exercise prescriptions, dietary guidance based on the Dietary Approaches to Stop Hypertension diet, medication adherence strategies, and regular blood pressure monitoring. Over a 12-week period, participants in the intervention group experienced significant improvements compared to the control group, including a reduction in systolic blood pressure by 7.36 mm Hg ($p$=0.002), increased exercise time, enhanced medication adherence, and more frequent blood pressure monitoring. Several other community-based intervention studies performed across the world including India, Mexico, Nepal, Brazil, and Kenya have addressed hypertension and/or other major CVD risk factors, including obesity, diabetes, and even depression.[40-45] The core strategies including utilization of community resources, cultural adaptations, and incorporation of innovative approaches such as faith-based engagement and digital health technologies are broadly applicable across these conditions and have been implemented.

It is worth mentioning that many community-based cardiovascular efforts originate organically within communities and operate independently of research protocols. For instance, since 2016, Linda Peterson, MD, a cardiologist at Washington University in St. Louis, has led a unique early prevention school-based cardiovascular outreach initiative targeting both grade school and high school students across the St. Louis region.[46] The program introduces young students – many from underserved communities – to the basics of heart function, the importance of cardiovascular health, and the role of cardiologists. Over the years, the program has expanded from a single-classroom effort to a multi-team initiative, reaching an estimated 1,300 students across several public schools. While not originally designed as a research study, this model demonstrates potential in academic-community partnerships aimed at improving cardiovascular health literacy from an early age. Such programs present valuable opportunities for systematic research, and efforts should be made to remove barriers and facilitate funding mechanisms that support their integration into formal study designs. If proven effective through research, these programs could be implemented either as a standalone intervention or integrated into broader, multimodal community-based strategies to improve cardiovascular health across diverse communities in the US and globally.

## 6. Future directions: 2025 and beyond

CVD community-based interventions are emerging as essential tools for reducing population-level risk, improving equity, and fostering sustainable health behavior change. Moving forward, the continued success and scalability of these interventions will depend not only on innovation and research but also on their thoughtful integration into healthcare systems and policy frameworks (Figure 1).

Digital health technologies offer immense potential for expanding the reach and efficacy of community-based interventions.[47] Incorporating mobile platforms, wearable devices, and app-based peer support has shown promise in improving blood pressure control, medication adherence, and patient engagement. Furthermore, the integration of artificial intelligence into community-based research offers a unique opportunity to leverage big data, particularly data on social determinants of health to design interventions, identify at-risk populations, and optimize resource allocation.[48] However, barriers such as digital literacy

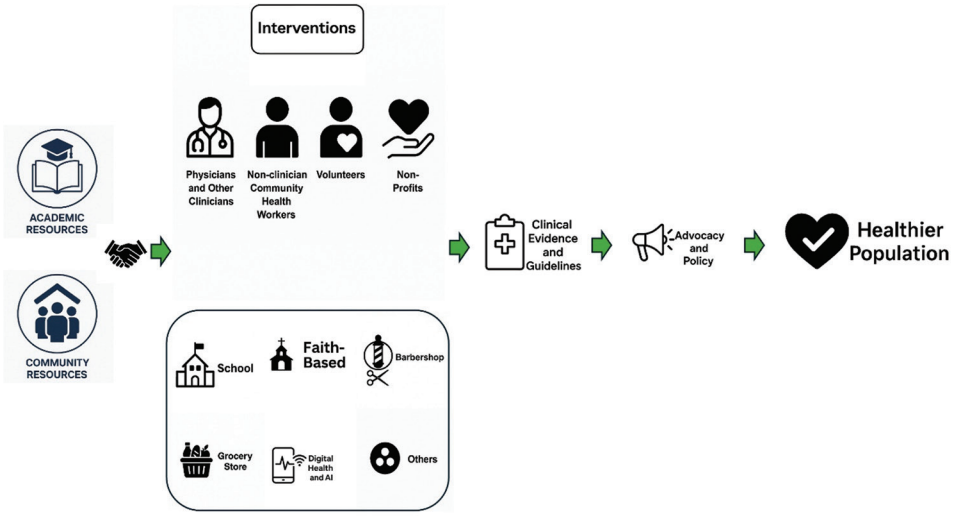


**Figure 1.** Pathway from academic and community resources to evidence, policy, and population health

gaps, inconsistent internet access, and device affordability continue to limit reach among those most at risk. Moreover, many digital interventions lack cultural considerations or real-world usability testing.

Future programs should prioritize inclusive design, multilingual interfaces, and user-centered development involving the target populations from the outset. They should also integrate community programming with standard pharmacologic and clinical care. While community-based CVD interventions have shown promise, many are limited by significant heterogeneity in methodology and a lack of integration with comprehensive clinical management. Few studies evaluate the impact of community-based interventions alongside a robust clinical care plan. To enhance effectiveness and real-world applicability, future research should prioritize clinical-community partnerships and design care pathways that span pre-treatment, concurrent treatment, and post-treatment phases, ensuring sustained lifestyle support across the continuum of care. The absence of standardized outcome measures, core implementation frameworks, and uniform reporting practices limits the ability to compare results across studies or conduct robust meta-analyses. Many promising programs remain unpublished or unevaluated due to resource constraints, limited research infrastructure, and challenges navigating institutional review board processes. Future efforts should prioritize the development of flexible yet rigorous evaluation models such as hybrid effectiveness-implementation designs[49] and the establishment of core outcome sets for community-based CVD research.

Furthermore, many community-rooted strategies remain disconnected from guidelines and the broader healthcare system. Successful integration requires robust evidence on the efficacy of the intervention, clear referral pathways, inclusion of community health workers in care teams, and bidirectional communication between clinical and community settings. Barriers such as limited reimbursement for non-clinical roles, poor electronic medical record data interoperability, and lack of institutional support continue to hinder progress. Addressing these challenges will require aligning community efforts with population health goals and value-based care metrics recognized by payers and accrediting bodies. Health policy will be essential in supporting this integration. Federal and state agencies can drive change through reimbursement models, workforce incentives, and investments in infrastructure. Policies that promote community health worker certification and fund grassroots innovation through research-practice partnerships will be key.

## 7. Conclusion

Community-based interventions have become essential in addressing the persistent burden and disparities of CVD. Programs grounded in cultural relevance, trust, and local infrastructure offer scalable solutions that extend beyond clinical settings. A growing body of evidence supports the effectiveness of these interventions across diverse populations and delivery models. As this evidence base grows, it is now imperative to integrate these approaches into mainstream healthcare, supported by sustainable funding, robust research infrastructure, and policy reform.

This conceptual framework illustrates how a partnership between academic institutions and community entities can contribute distinct but complementary resources that feed

into the design and delivery of effective community-based cardiovascular interventions. When rigorously studied, these interventions can generate evidence that informs clinical guidelines, shapes policy, and ultimately drives improvements in cardiovascular health at a population level.

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ACCSCIENCE PUBLISHING

Global Translational Medicine

PERSPECTIVE ARTICLE

# Personalized and precision medicine as a health-care model of the next step generation through translational applications of individualized nutrition- and food design-driven resources

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## Abstract

In the modern era, health is a key element in shaping the trajectory of human civilization. The personalized and precision medicine model is a preventive strategy capable of operating across a wide range of technological applications, from biomarker-driven genomic profiling to determine the characteristics of an individual's genomic landscape, to combinatorial assessments of the individual's interaction with the microenvironment for constructing the individual phenotype. Omics technologies demonstrate their multidisciplinary potential in pre-clinical

screening, predictive and prognostic diagnosis, multidimensional monitoring, and targeted therapy, through the prism of preventive and curative rehabilitation strategies concerning a particular individual, as well as the principles and resources of genomic biostatistics and molecular epidemiology at the population and national levels. Therefore, this article aims to highlight the omics approach to nutritional assessment and dietary recommendations from the individual to the population level.

***Keywords:*** Omics technology; Genetics; Proteomic; Metabolomic; Biomarkers; Interactomics

## 1. Introduction

The progress of health-saving technologies has substantially transformed modern health care, presenting personalized approaches that are both pioneering and effective. Among these, personalized nutrition (PN) and precision foodomics (PF) have emerged as significant fields, taking advantage of the power of omics technologies to provide tailored nutritional advice and interventions. This personalized approach is critical for addressing the unique health needs of individuals, enhancing overall well-being, and preventing nutrition-related diseases. In this context, the main objective of PN and PF is to promote and improve health by utilizing omics, phenotypic, medical, nutritional, and other relevant personal data to provide more precise dietary guidance and customized nutritional products and services. These approaches can be applied to patients, individuals at risk, and healthy individuals, regardless of whether they have a genetic predisposition to specific disorders, including metabolic and nutritional conditions[1-8] (Figures 1 and 2).

Health-saving technologies aim to prevent and delay illnesses, improve well-being, and reduce health-care needs, with nutrition being a key determinant of individual health outcomes. Diets can increase disease risk or promote better health depending on their genetic impact. Nutrigenomics explores the connection between food and genetics and facilitates innovations in health care. Developments in wearable devices and artificial intelligence (AI)-assisted diagnostics empower individuals and enable individualized, patient-centered care. These technologies help improve health-care access and ensure quality medical expertise is available to all. By adopting these innovations, we can transform health care and build a healthier, more resilient world for everyone.

## 2. Omics technologies: The new generation of health-saving technologies

The development of multiomics technologies, which include transcriptomics, proteomics, epigenomics, metabolomics, and microbiomics, has significantly enhanced our ability to interpret genomic data and improve health outcomes. By integrating these interconnected datasets, integrative multiomics offers a more comprehensive understanding of human health and disease. Health-saving technologies, embedded within the infrastructure of the model of personalized and precision medicine (PPM), represent a unique preventive strategy (Figures 3 and 4).

Multiomics technologies operate with large amounts of data to identify the relationships between biological processes at various levels, the dynamics of borderline states with induction of pathological processes, and the development of optimal protocols for targeted therapy. With the introduction and study of systems omics technologies, the mechanisms of induction, development, and progression of chronic health conditions, including nutritional and hereditary conditions, have moved from hypothetical approaches to diagnosis, treatment, and prevention to integrated strategies[16] (Figures 5 and 6).

Omics technologies, including genomics, proteomics, metabolomics, and microbiomics, are at the forefront of PPM. These technologies offer an in-depth analysis of biological molecules, revealing intricate interactions among genes, proteins, and metabolites. By integrating multiomics data, researchers and health-care providers can enhance their understanding of an individual's overall health condition, facilitating precise and personalized interventions. The omics technologies applied in PN are particularly transformative. Nutrigenomics, a subfield of omics, studies how food affects gene expression and how genetic variations alter the body's response to nutrients. This knowledge allows for the development of dietary proposals tailored to the genetic predispositions of each individual, improving health outcomes and preventing chronic diseases. Several fundamental areas of omics technology and their potential applications are discussed in the following sections.

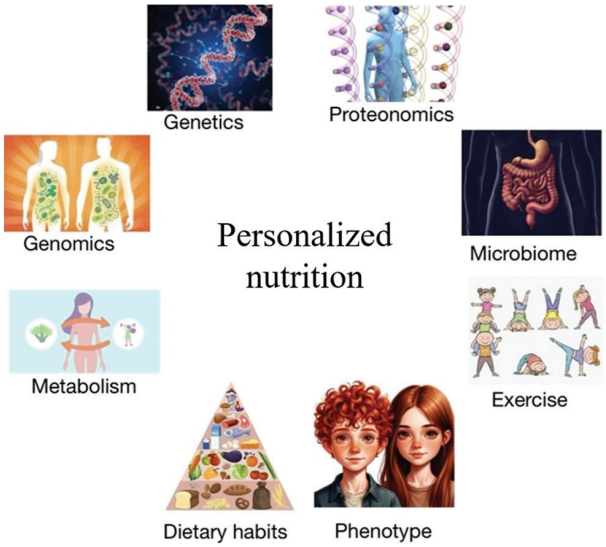


**Figure 1.** Personalized nutrition includes several factors, such as the genome, metabolome, microbiome, lifestyle, diet, and phenome. Personalized nutrition utilizes advanced analytical technologies to efficiently manage and provide detailed information about individuals' genetics, metabolomes, microbiomes, and phenomes. Within this paradigm, the integration of advanced omics technologies with comprehensive phenotyping has the potential to reveal previously undiscovered hereditary factors and gene–environment interactions.[9,10]

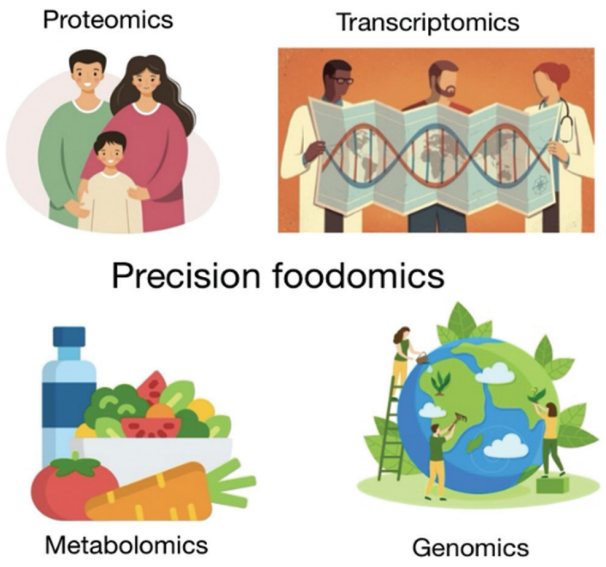


**Figure 2.** Precision foodomics is a new discipline that was introduced as a global strategy through the application of advanced omics in the food science domain. It examines the food and nutrition domains through the application and integration of advanced omics technologies. Precision foodomics is already a widely used methodology in food science analyses. Both targeted and non-targeted approaches using transcriptomics, proteomics, metabolomics, and genomics are discussed, along with an overview of data integration in multiomics datasets to fully interpret the results from a global precision foodomics perspective.[11,12]

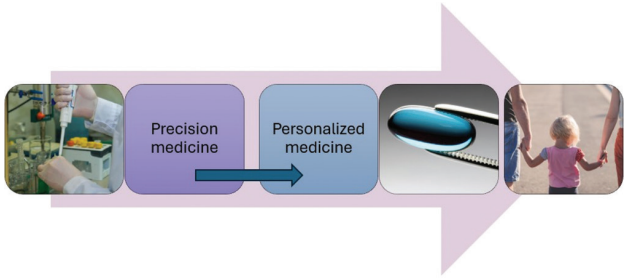


**Figure 3.** A basic framework of information technology-integrated PPM. Precision medicine identifies differences among individuals, categorizing them based on environmental, biological, and psychosocial factors. Personalized medicine takes these differences and implements prevention and treatment tailored to each individual. Powered by high-throughput omics technologies and computational capabilities, PPM provides multi-scale, in-depth insights into cells, organisms, and populations. By leveraging these conceptual and technological advancements, PPM is built on two core pillars: Data generation and data modeling. High-throughput omics technologies facilitate the acquisition of comprehensive and holistic biological information, while computational advancements enable high-dimensional data modeling, making the analysis both accessible and user-friendly. The current focus on biologic omics in discussions of PPM should not divert attention from traditional approaches to personalized care, including clinical evaluation, the importance of clinician–patient rapport, and addressing social determinants of health and lifestyle behaviors. To achieve further improvements in health care, progress on all of these fronts must continue, not solely in omics-based PPM.[13]
Abbreviation: PPM: Personalized and precision medicine.

### 2.1. Genomics

PPM adapts therapies, disease prevention, and health maintenance to meet patients' unique needs. Various therapy types—such as proteins, nucleic acids, viruses, cells, genes, and irradiation—can benefit from genomics. This shift expands the importance of pharmacogenomics and nutrigenomics in medicine. PPM seeks to enhance patients' health care by utilizing predictive genomic biomarkers with the aim of improving patient outcomes and minimizing the risk of adverse effects[19,20] (Figure 7).

Metabolic and nutritional disorders are increasingly prevalent worldwide. PPM has the potential to address a wide range of illnesses and equip physicians with the tools to predict the most effective treatment for patients with metabolic disorders or implement preventive measures for individuals at risk. Identifying key diagnostic and predictive biomarkers is essential for developing targeted treatment plans for metabolic and nutritional diseases, using a comprehensive analysis of metabolomic, proteomic, genetic, and clinical data. To achieve this, real-time modeling of clinical data alongside multiple omics datasets is crucial, as it helps uncover underlying biological mechanisms, risk factors, and other valuable information that support early diagnosis and prevention of chronic or complex diseases. Integrating advanced technologies such

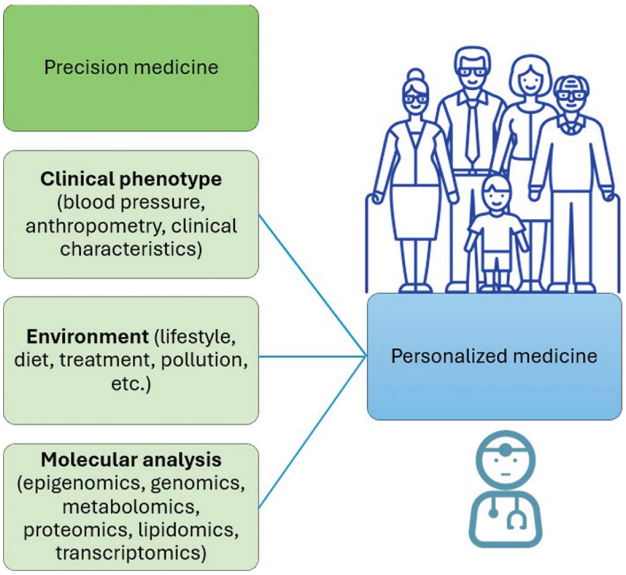


**Figure 4.** PPM represents an ambitious challenge for medicine and health-care services, aiming to ensure targeted care pathways through more personalized approaches from the outset. PPM has emerged as a prominent topic across various research fields and is likely to play a crucial role in the future. The growing interest in this area can be attributed to the advancements in systems biology and high-throughput technologies. Notably, the expanding knowledge and improved interpretation of genetic data will deepen our understanding of physiological processes in health and disease, paving the way for more precise diagnoses and personalized treatment. This approach can also help reduce the burden of disease by enhancing prevention and treatment strategies through the integration of multiple data sources. Furthermore, PPM seeks to lower health-care costs and minimize adverse events by optimizing the selection of the right therapy at the right time for each patient. Successfully implementing PPM into clinical practice requires a comprehensive, multi-level approach to patient care. At the molecular level, the multiomics approach—including transcriptomics, metabolomics, genomics, proteomics, and epigenomics—offers a deeper understanding of patient conditions, from the underlying causes of diseases to their functional consequences. This information should be integrated with the study of the "exposome," which encompasses the totality of an individual's lifetime exposures and their impact on health. By combining these insights with clinical patient data, physicians can develop personalized therapies tailored to each individual.[14,15]
Abbreviation: PPM: Personalized and precision medicine.

as AI and machine learning (ML) is vital for consolidating diverse data, analyzing multiple variables, building clinical biomarker databases to aid decision-making, and creating ethical protocols to address these challenges.[21,22]

In recent decades, genome-wide association studies (GWAS) have been employed to identify the genetic foundations of chronic diseases, uncovering the impact of various common genetic variants on disease risk. These disorders include—but are not limited to—cardiovascular disease (CVD), cancer, metabolic, neurodegenerative, and neuropsychiatric ailments. Nonetheless, despite the significant hereditary component observed in these chronic illnesses, the genetic variants identified explain only a small portion of disease variability. These genetic variants typically have a small effect, contributing to disease treatment models via polygenic risk scores (PGS), also known as polygenic risk indicators. PGS are quantitative factors that capture the cumulative influence of several common genetic variants on a specific condition or illness. Calculated as the sum of the risk alleles in an individual, PGS are weighted according to the effect sizes of these alleles, as estimated by independent phenotype-training GWAS. Thus, PGS assesses a person's genetic predisposition to a trait or disease, based on their genotype profile and using independent GWAS information as a learning model.[15,23]

There is a strong rationale for integrating PGS with other risk algorithms incorporating environmental components to forecast the risk of chronic diseases in routine clinical practice. For example, CVD has a substantial dataset with the potential for cost-effective application of PGS.[15,23] However, current dietary guidelines for CVD remain a topic of debate. A pooled analysis involving 172,891 participants revealed 9,453 cases of coronary heart disease (CHD) and 8,182 cases of stroke. In addition, an updated meta-analysis drew evidence from 49 previous non-overlapping studies, which showed varying associations for different types of saturated fatty acids (SFAs). Even-chain SFAs were positively associated with CVD risk, whereas odd-chain and longer-chain SFAs had a negative association. Overall, higher total levels of n-3 polyunsaturated fatty acids (PUFAs) were linked to a lower risk of CHD, whereas higher total n-6 PUFAs were associated with a reduced risk of stroke. When examining individual PUFAs, linoleic acid—the predominant n-6 PUFA—along with docosahexaenoic acid and n-3 docosapentaenoic acid, was negatively associated with the risks of CHD and stroke. In contrast, dihomo-γ-linolenic acid was positively associated with both diseases. Interestingly, α-linolenic acid, an n-3 PUFA mainly found in plant sources, did not show a relationship with lower risks of CHD or stroke. Furthermore, arachidonic acid, a key metabolite of linoleic acid, was not linked to an increased risk of either condition.[24]

Although 30–50% of common cancers are attributable to lifestyle and environmental factors, cancer remains a considerable global health burden, with 20 million new cases in 2022. Prevention is the most effective and economical strategy for cancer control, underscoring the need for tools that support public adherence to preventive guidelines. Adherence to the World Cancer Research Fund and the American Institute for Cancer Research's Cancer Prevention Recommendations is associated with

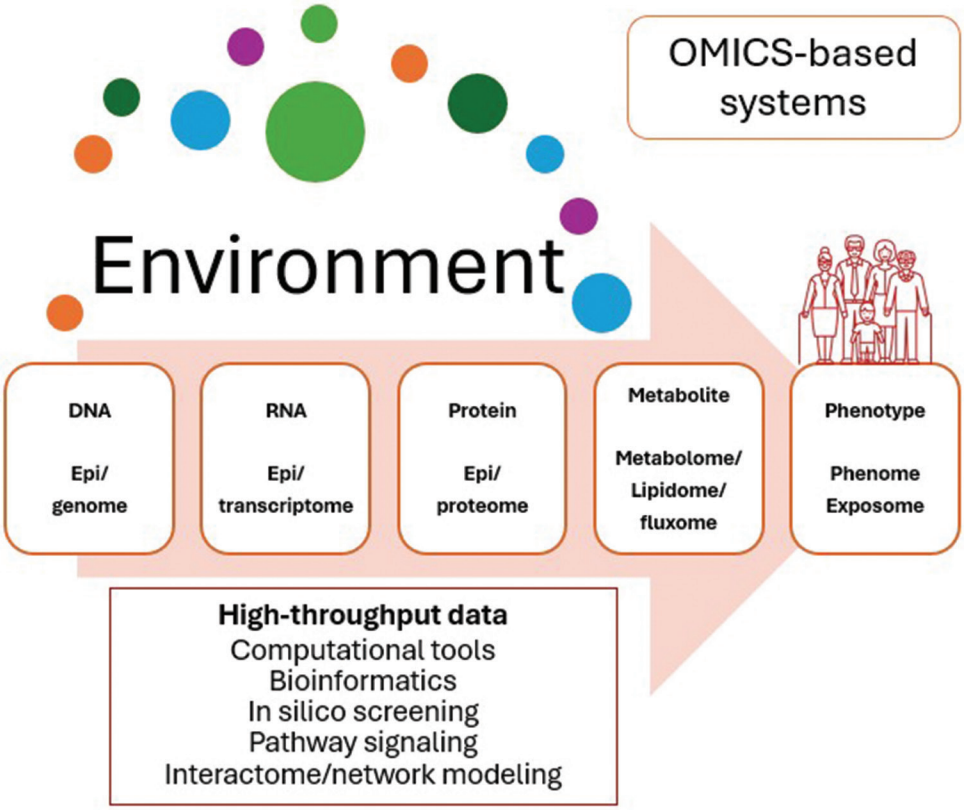


**Figure 5.** Building blocks of the omics approach in PPM. Omics technologies are the cornerstone of PPM, encompassing high-throughput analyses such as genomics, transcriptomics, proteomics, and metabolomics/lipidomics. These approaches, when integrated with robust systems biology, bioinformatics, and computational tools, enable the comprehensive study of cellular mechanisms, interactions, and functions across tissues, organs, and the entire organism. By operating at the molecular level in a non-targeted and unbiased manner, omics technologies provide a deeper understanding of biological complexity and disease processes.[17]
Abbreviation: PPM: Personalized and precision medicine.

a lower risk of cancer and better survival rates. However, no rapid, validated tools currently exist to assess individual adherence. To address this, Chaplin *et al.*[25] developed and validated a 13-item screening tool that evaluates compliance with seven of the 10 recommendations issued in 2018 by the World Cancer Research Fund and the American Institute for Cancer Research, with the assessment taking <6 min to complete. The tool provides both overall and recommendation-specific scores (met, partially met, and not met), facilitating targeted prevention strategies. This tool supports the implementation of comprehensive lifestyle interventions that lower the risk of cancer and other chronic diseases.

Nevertheless, clinical recommendations for primary prevention do not suggest using genetic information to assess risk. This is because none of the PGS with high predictive power have demonstrated the ability to modify treatment in a cost-effective manner or encourage patients to change their lifestyles.[15,23] Although most relevant and recent studies conducted in developing countries have shown a significant improvement in net risk reclassification, a cost-benefit analysis is required to corroborate its clinical usefulness in risk assessment. Several factors must be considered essential, including the number of participants, the expenses of assessing health indicators, and the management of false positives. Furthermore, significant challenges include the prohibitive cost of laboratory testing and the relatively modest increase in predictive accuracy.

In addition to authorized translational genomics and pharmacogenomics-driven approaches, nutrigenetics and nutrigenomics, along with other omics technologies, are becoming increasingly essential in PN-based care to understand how an individual responds to nutrition-driven therapy. Nutrigenetics and nutrigenomics, along with regular omics tools, serve as instruments to create goals for more accurate nutrition evaluation. They offer valuable insights into molecular mechanisms, as individual nutritional needs can differ greatly. Omics testing reveals modest individual variability and is essential for leveraging this data in PN development. While diet-based therapies

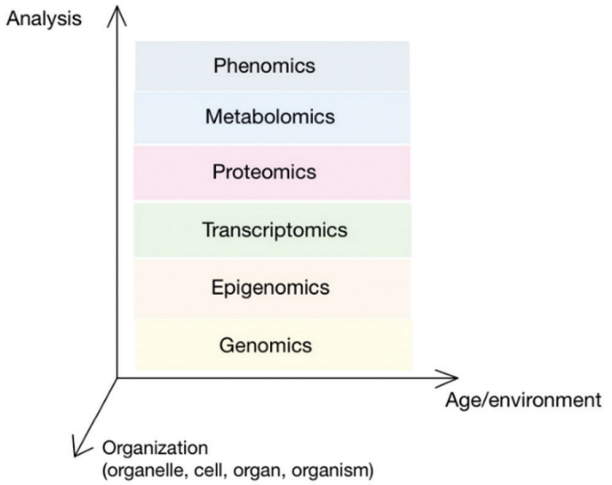


**Figure 6.** Multiomics data encompass information from various omics disciplines, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and phenomics. These data are derived from diverse assays and experiments, spanning multiple spatial and temporal scales. While genomics has traditionally been a primary focus in personalized and precision medicine, other omics fields are increasingly contributing to a more comprehensive understanding of how an individual's complex biology influences their health. High-throughput multiomics technologies enable the collection of extensive and holistic biological data, whereas advanced computational tools facilitate high-dimensional data modeling, making analysis accessible and user-friendly.[18]

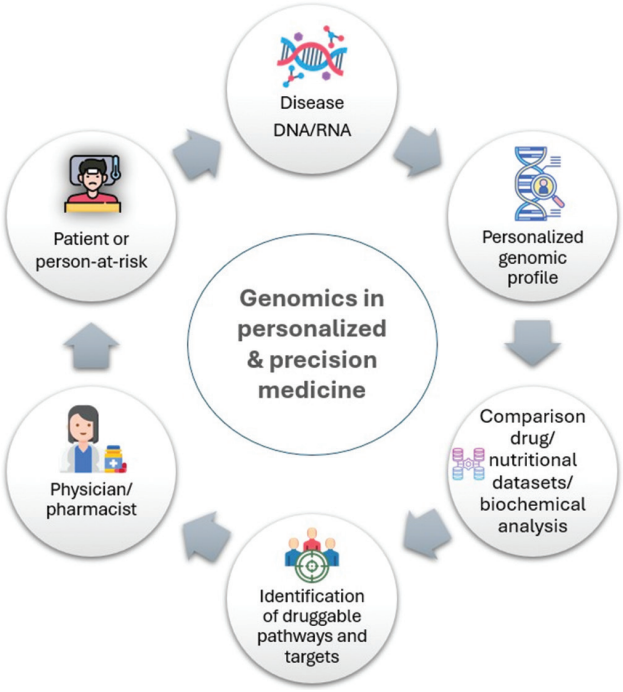


**Figure 7.** Genomics through the multipoint view of personalized and precision medicine. Genomics provides valuable biological insights, as it is a branch of life sciences focused on understanding and mapping genomes.

are used for various clinical conditions, such as inborn errors of metabolism, progress in expanding omics data has been limited. This hampers a deeper mechanistic understanding of cellular networks influenced by nutrition-driven gene expression and complete gene regulation. The main challenge lies in the clinical sector, which should integrate existing data, overcome the limitations of self-reported procedures in research, and make omics data, along with nutrigenetics and nutrigenomics research, widely accessible.[20,26-29]

We have only recently started to recognize specific gene–diet connections, as many clinical and molecular phenotypes, such as body mass index, are influenced by multiple genes. While nutrigenetics focuses on how genetic variations impact metabolism, nutrigenomics examines how nutrients (food compounds) affect gene activity, assessing how mutations affect the assimilation of metabolites. Recent advancements in genomics and PPM have led to a growing number of evidence-based applications with the potential to significantly reduce morbidity and mortality in millions of individuals.

In summary, it is important to note that, in contrast to general genomics-related achievements, nutritional genomics is still in its early stages compared with PPM. However, using genomics indicators and other clinical tools represents a practical application of this emerging technology. Meanwhile, advancements in genomics have led to the concept that a deeper understanding of individual characteristics, such as genotype, can enable more precise personalization of pharmaceutical and nutritional therapies. PN is customized based on an individual's specific genetic profile, lifestyle, and health objectives, in contrast to general dietary guidelines that offer broad recommendations for the population as a whole[30] (Figure 8).

It is crucial to recognize that current PN approaches have achieved only limited scientific success in improving dietary habits or addressing diet-related health conditions. These strategies often target narrow population subgroups, limiting their broader impact on public health. To overcome this, a more holistic approach is needed—one that integrates biomedical and dietary assessments with psychobehavioral insights and innovative digital and diagnostic technologies for comprehensive data collection. An adaptive PN counseling system addresses this need by combining biomedical and health phenotyping, stable and dynamic behavioral indicators, and contextual food environment data. This integration utilizes advanced digital tools, including sensors and AI-driven methods. Such a system holds significant promise for transforming individualized nutrition strategies into scalable, accessible

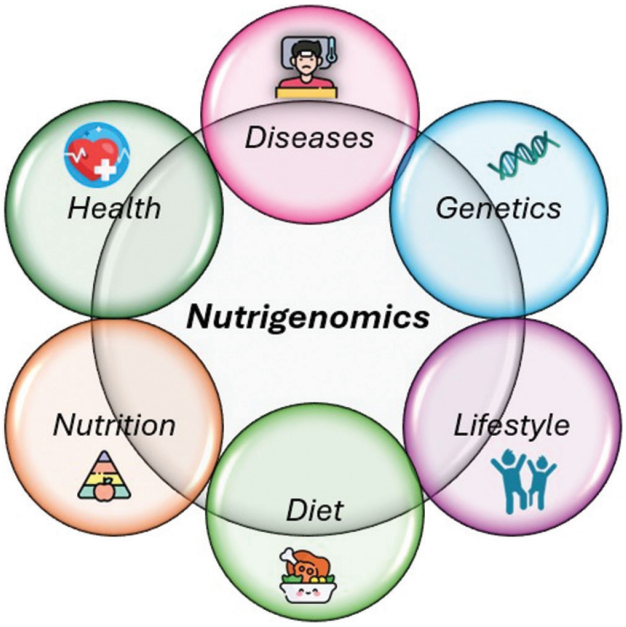


**Figure 8.** The evolution of personalized nutrition. Several omics factors can influence an individual's response to diet and its impact on health. The emergence of new nutritional biomarkers, which integrate information on intake and its effects on the organism, has generated interest, especially regarding their relationship with health and disease. Omics technologies play a crucial role in exploring these connections. At its core, personalized and precision medicine represents the intersection of individuals, their environment, and the evolving markers of health and disease, along with the social and behavioral factors that shape outcomes over time. This includes (i) people: Patients, individuals at risk, and populations served; (ii) markers: Indicators of health and illness, encompassing genetics, genomics, metabolomics, phenomics, pharmacogenomics, and other omics platforms; (iii) exposome: Environmental exposures and influences, both internal (e.g., microbiome and gut–brain interactions) and external (e.g., socioeconomic factors, food quality, agriculture, and geographical influences); and (iv) behavioral health: Factors such as exercise, self-care, addiction, anxiety, and lifestyle choices that impact individuals and populations. Together, these elements form the foundation for understanding health and disease through a personalized, multidimensional approach.[31]

solutions that deliver meaningful health benefits to a large population.[32]

It is essential to consider both ends of the spectrum—nutrigenomics and deep phenotyping—while accounting for multiple factors when designing personalized and unbiased nutritional solutions for individuals or specific population subgroups. In addition, a collaborative effort between basic scientists, clinical researchers, and health-care professionals is essential to create a comprehensive foundation that enables the successful implementation of these novel insights at the population level. This study explores the latest advancements in analyzing and monitoring dietary habits, food behaviors, and deep phenotyping. In addition, we highlight the relevance of emerging applications in conjunction with nutrigenomics, proteomics, and metabolomics.

Today, genomics-driven PN accounts for almost all relevant environmental influences throughout life on human health conditions. The latest advancements show that genetic variation affects connections between nutrients and the genome, which may alter disease risk. While there are food products that cater to the needs or preferences of specific consumer groups, these choices are mainly driven by empirical consumer science rather than by nutrigenomics and nutritional approaches. This understanding helps elucidate human variability in dietary preferences, requirements, and responses, paving the way for future scientific tools for consumption assessment, guided by PN-based advice for health maintenance and disease prevention.[33]

### 2.2. Proteomics

As mentioned previously, systems biology integrates multidisciplinary scientific approaches to predict and describe the dynamic properties of living biosystems, which are understood as complex signaling networks. In the realm of nutritional sciences, systems analysis of both standard and nutrient-modified signaling networks, along with an understanding of underlying genetic polymorphisms, is expected to pave the way for a future where individual health is enhanced through predictive and preventive nutrition.[34]

The advancement of omics technologies has provided effective methods for a thorough and targeted study of biological systems, leading to more precise diagnostic tools and the development of targeted treatments. Advances in proteomics technology allow for the simultaneous identification and quantification of the expression profiles of thousands of proteins. Proteomic analysis has emerged as a powerful technique for describing the patients' molecular profiles, allowing analysis of tissue and bodily fluids.

Proteomics plays a critical role in discovering novel biomarkers for disease diagnosis, prognosis, and therapeutic strategies. Identifying and quantifying proteins provides crucial data about disease conditions and how they respond to treatment. More broadly, proteomics can uncover the molecular mechanisms critical for an organism's adaptation to its ecological niche.[35] In this context, proteomics involves the large-scale study of proteins, focusing on their structures, functions, and roles in living organisms. As such, proteomics is increasingly used to explore both health and disease, as well as to address issues related to food quality, safety, bioactivity, and to develop healthier food products.

The goal of clinical proteomics is to identify and quantify proteins in clinical samples such as blood, urine,

or tissue, with the aim of gaining insights into disease mechanisms, discovering biomarkers for disease, and pinpointing potential therapeutic targets for disease prevention[36-38] (Figure 9).

Nutritional proteomics (Figure 10), also known as nutriproteomics, leverages proteomic tools to analyze molecular and cellular changes in protein expression and function while assessing how proteins interact with food nutrients.[41] It is essential to recognize that proteins specific to an organ, once released from damaged tissue, often undergo degradation upon entering the bloodstream. In addition, blood serum contains many proteins, including albumin, which has a broad dynamic range, making accurate quantification challenging. Variations in serum protein levels can reflect shifts in the inflammatory response, offering insights into the underlying pathological process. Therefore, collecting organ-specific fluids such as synovial fluid, urine, or spinal fluid near the affected tissue may provide a more reliable source of potential diagnostic and therapeutic biomarkers than serum. Protein profiles have been shown to serve as valuable markers, capable of predicting treatment outcomes for various diseases, including nutritional disorders. The standardization of methodologies and integration of proteomic data into publicly accessible databases are beginning to address these challenges. With these advancements, the era of personalized, patient-tailored therapeutic strategies is approaching. As a result, clinical and nutritional proteomics seek to apply the methodologies and principles to the fields of PPM and PPM-guided PN and PF.[40]

Comprehensive protein profiles complement the genome by reflecting expression in specific cells or organs, plasma, or serum, enabling the identification of biomarkers that respond to dietary changes or treatment and potentially predicting biological processes. While proteomics has yet to be widely adopted in nutritional research, it offers advantages over transcriptome analysis, as it directly examines the molecules responsible for conducting biological functions.[42]

In the food industry, proteomics-based techniques play a critical role in ensuring food safety and authenticity. These methods are used to detect foodborne pathogens by analyzing variations in their proteome, identify allergens and toxins, validate and optimize food processing, and pinpoint bioactive compounds in functional foods. In addition, proteomics enables the authentication of meat and

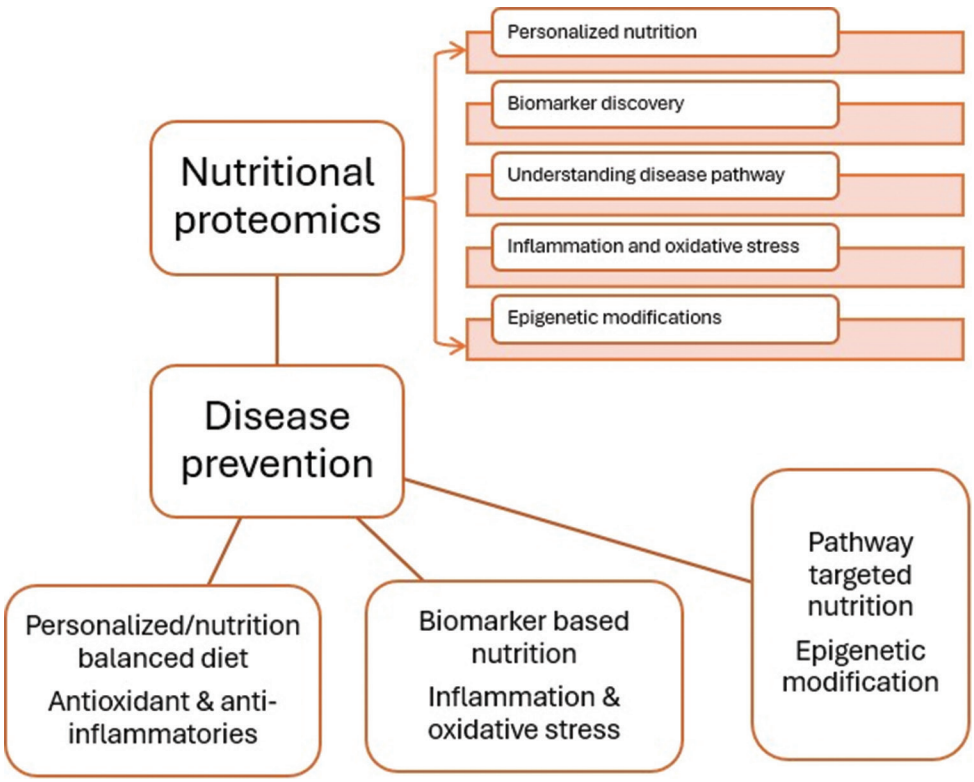


**Figure 9.** The intersection of nutritional proteomics and disease prevention. Personalized nutrition is a powerful tool that enables consumers to improve their dietary habits, optimize health, and prevent diet-related diseases. Omics technologies provide detailed insights into metabolic dynamics. However, translating these insights into personalized, simple, and affordable nutrition protocols remains challenging due to the complexity of metabolism and technical and economic limitations. Proteomics is essential to identify proteins that serve as biomarkers associated with disease progression.[38,39]

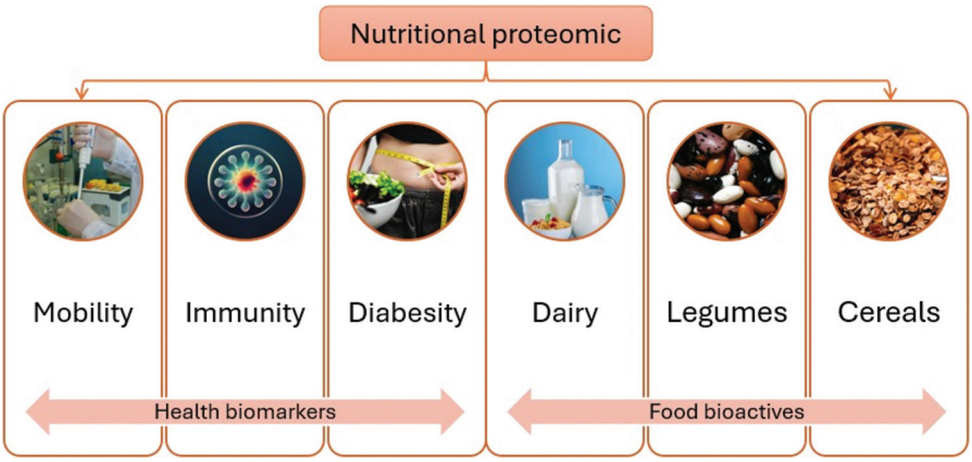


**Figure 10.** Nutritional proteomics (nutriproteomics) focuses on two key outcomes: (i) Identifying health-related biomarkers and (ii) studying food bioactives, along with their applications. The emerging field of nutriproteomics, which holds great promise in nutrition science, builds upon nutrigenomics and nutrigenetics while serving as a complementary approach. It plays a crucial role in molecular nutrition research and personalized nutrition by achieving two main objectives: (i) Analyzing and quantifying bioactive proteins and peptides derived from food and (ii) identifying biomarkers that reveal mechanisms of action, efficacy, and potential side effects of nutritional interventions. Modern nutrition research aims to promote health, prevent disease, enhance performance, and evaluate risks and benefits. As a result, nutriproteomics must identify early pre-symptomatic biomarkers to detect subtle metabolic deviations, though a clear definition of "normal" metabolism remains lacking. From a molecular standpoint, nutriproteomics encompasses two key areas: (i) The discovery, quantification, and characterization of biomarkers and (ii) the analysis of bioactive compounds, with proteins playing a significant role in nearly all biological functions. These biomarkers must be integrated with genomic and genetic markers, as nutrition aims to optimize specific health aspects without negatively impacting others. Therefore, holistic and integrative approaches are essential. The advancement and effectiveness of proteomics in nutrition and health will depend on several critical factors, including study design, data generation, data processing, data correlation, genetic susceptibility, and epigenetics.[40]

dairy products by identifying species-specific biomarkers. With its broad application in food safety, nutrition, and related fields, proteomics holds great promise for the future. Moreover, by examining how different processing techniques affect food proteins, proteomics contributes to enhancing food quality and refining production processes.[43]

Nutriproteomics applies proteomic methods to nutrition research and practice. It also represents the interconnection of bioactive food ingredients with proteins through two basic approaches. The influence of nutrients on protein expression is assessed through protein mapping and by examining nutrient–protein interactions, including post-translational modifications and small-molecule binding. Integrating these insights with functional data from established biochemical and physiological methods enhances our understanding of how bioactive dietary components contribute to diet-related disorders, such as diabetes and obesity. These biomarkers serve as valuable tools for disease diagnosis and treatment, offering precise indicators of the efficacy and safety of specific nutrients.[37,41,42,44-48]

The field of nutrition research has been transformed by proteomics as a powerful discovery tool. Proteome analysis is a valuable tool for addressing key nutrition-related disorders.[49] When integrated with other advanced omics technologies and biological systems, proteomics can greatly enhance the identification of key proteins that regulate metabolic pathways. The synthesis, degradation, and modifications of these proteins are influenced by specific nutrients and dietary factors.[50] This innovative approach will accelerate our understanding of the intricate mechanisms governing nutrient utilization, including dietary efficiency, facilitate the identification of novel indicators for nutritional status and disease progression, and establish a framework for dietary prevention and intervention in disease. Therefore, proteomic analysis promises to improve human health and increase the efficiency of livestock farming.

The evolution of PPM relies on comprehensive and integrative approaches (e.g., proteomic technologies) to provide better patient care and valuable disease insights. This application of proteomic methods to diseases is known as clinical proteomics, a powerful tool that studies changes in disease pathways and identifies new protein biomarkers. A proteomic biomarker is a specific peptide or protein associated with a specific condition, such as the onset or progression of a disease or response to treatment, or a measurable characteristic that serves as an indicator of normal biological processes, pathogenic processes, or responses to therapeutic interventions.

Proteomics is thus becoming a powerful tool in the realm of PN and PF, offering insights into an individual's

metabolic state, nutrient deficiencies, disease risk, and optimal dietary needs. As technology continues to advance, proteomics analysis will become increasingly feasible and affordable, paving the way for PN to become a standard approach to health care. Proteins and metabolites linked to dietary patterns may serve as prognostic markers, guiding future clinical interventions and aiding in the identification of intermediate phenotypes while also shedding light on the molecular mechanisms underlying diet-related disease. However, there is limited data on the proteomic and metabolomic signatures of healthy dietary patterns.

### 2.3. Metabolomics

Individuals have varying needs for and responses to nutrients and bioactive molecules in their diet. At the same time, biological systems are highly complex, with essential processes occurring at multiple molecular levels, including nucleic acids, proteins, and small molecules. In this context, metabolic heterogeneity is influenced by numerous factors, such as genetic and epigenetic variations, the microbiome, lifestyle, dietary intake, and environmental exposures.[3,51-54]

Metabolomics is the scientific study of chemical processes involving metabolites—small-molecule substrates, intermediates, and end products of cell metabolism found in tissues, cells, or biofluids. Unlike genomics, transcriptomics, or proteomics, which typically rely on a single instrument for measurements, metabolomics necessitates a diverse set of analytical tools.

Targeted metabolomics enables the identification of specific metabolites through comparison with established chemical parameters, facilitating the development of biomarkers and the testing of hypotheses. However, untargeted metabolomics focuses not on the characterization and quantification of compounds but rather on the identification and discovery-based research. It can identify and quantify bioactive compounds, but has both advantages and disadvantages.

Nutritional metabolomics is a powerful and precise approach for identifying and characterizing biochemical pathways. It provides deep insights into the complex interplay between dietary exposure and chronic diseases, shedding light on metabolic phenotypic changes and their underlying mechanisms. This method has four main applications in nutritional research:

(i) Identifying dietary biomarkers.
(ii) Characterizing diet-related diseases and disease biomarkers.
(iii) Utilizing PN to elucidate mechanisms underlying dietary interventions.
(iv) PN and PF.[54,55]

These areas primarily focus on understanding how dietary compounds and their metabolites affect the host over time after consumption. They also aim to find biomarkers related to diet and determine how the dose of phytochemicals influences the connection between diet and health. Furthermore, metabolites serve as crucial biological communication channels, offering a valuable functional readout at the intersection of various influential factors that shape health and disease (Figure 11).

In the field of food science and nutrition research, there is an increasing emphasis on quantitative metabolomics. Targeted quantitative metabolomics is extensively applied in food composition analysis, body fat index characterization, detection and monitoring of nutrient deficiencies and metabolic disorders, dietary intake assessment, and the formulation of dietary guidelines for the prevention and treatment of chronic diseases.[28,57] The application of PN and PF in metabolomics can generate valuable data for optimizing nutritional regimens, supporting optimal child growth, and enhancing the composition of commercial products. These are just a few areas where metabolomics is making an impact, contributing to the advancement of personalized and precise health care. With current knowledge, metabolomics can be integrated into routine clinical practice. Sensitive metabolomic biomarkers may be detected using cost-effective and accurate test strips,

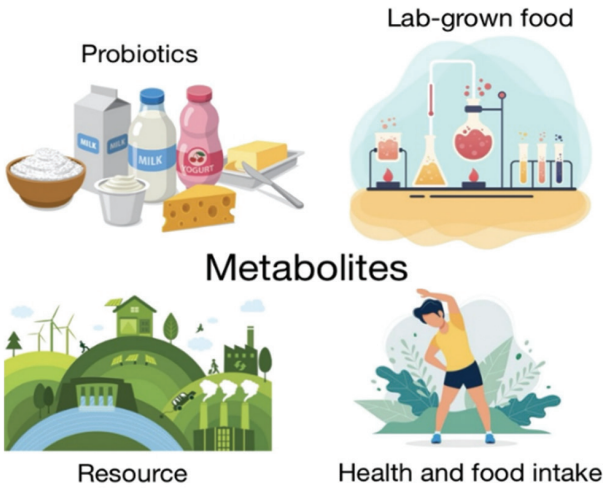


**Figure 11.** Metabolomics (food metabolome) via the phenotype of individuals and food. The food metabolome provides valuable insights into the relationships between metabolites, health, and nutritional status. It is a subset of the human metabolome, originating from the digestion and biotransformation of food and its components. While over 25,000 compounds have been identified in various foods, along with their metabolism by host enzymes and gut microbiota, the food metabolome remains highly complex, with its composition varying significantly based on dietary intake. This variability serves as a valuable and extensive data source for monitoring dietary exposure and identifying foods that influence disease risk.[54,56]

enabling rapid assessment of biofluids at the patient's bedside. This approach could improve diagnosis, treatment, and prognosis, paving the way for more personalized medicine.[31,58]

In this context, PN and PF are emerging branches of nutrition science that leverage omics technologies (e.g., genomics, proteomics, and metabolomics) to analyze individual responses to foods or specific dietary patterns. Their goal is to identify the most effective diet or lifestyle interventions for preventing or treating specific diseases. Metabolomics plays a fundamental role in nearly every aspect of PN and PF. It enables the comprehensive identification of thousands of compounds in foods, the detection of food byproducts in human biofluids or tissues, the assessment of nutrient deficiencies or excesses, the monitoring of biochemical responses to dietary interventions, and the evaluation of both short-term and long-term dietary habits. In addition, it aids in the development of targeted nutritional therapies. As a result, metabolomics is crucial to advancing nutritional science and making the implementation of PN and PF a reality.

In general, metabolic homeostasis is altered in critically ill patients. Maintaining homeostasis under continuously changing conditions is called phenotypic flexibility or systems flexibility. Under conditions of continuous energy overload, maintaining homeostasis has an adaptive cost. Adipose tissue stores excess energy. Nevertheless, when storage surpasses normal physiological limits, insulin resistance occurs, leading to complications such as ectopic adipose deposition in and around vital organs. This also causes elevated plasma glucose levels, which contribute to oxidative damage in the microvasculature and sustained low-grade inflammation caused by macrophage infiltration in adipose tissue.

A lack of phenotypic flexibility can lead to the development of pathologies or suboptimal health. However, pathology does not always emerge during the disease process or within the organ that loses flexibility. For instance, the inability of peripheral adipose tissue to effectively absorb glucose or convert it into fatty acids can result in the accumulation of lipids in the liver. This can also lead to insulin resistance in other organs, such as skeletal muscle and the liver. Factors that can trigger insulin resistance include poor nutrition, such as overnutrition or, in some cases, micronutrient deficiencies, and underlying diseases. In these situations, macronutrients and micronutrients act as key environmental factors influencing metabolite production through their effects on genetic regulation. In critically ill patients, where a comprehensive profile of circulating metabolites is analyzed, metabolomic studies consistently reveal that alterations in fatty acids, lipids, and tryptophan metabolite pathways are common and closely linked to disease states and outcomes.

To summarize, system flexibility plays a crucial role in health, disease, and potentially aging. However, interindividual variations arise from multiple factors and have diverse consequences. Systems flexibility integrates all interacting systems, each influenced by genetic components and environmental (exposomal) factors. Therefore, it is essential to not only observe and quantify individual parameters but also assess and intervene at the systemic level. Metabolomics examines shifts in an organism's metabolic state due to factors such as drug treatment, environmental influences, nutrition, genetic variations, toxins, and diseases. This is achieved by globally or comprehensively identifying and quantifying metabolites within biological systems.

Given the strong association between nutrition and most chronic diseases, nutritional metabolomics holds significant promise for elucidating the relationships among disease, nutritional status, nutrient intake, and diet. This approach involves examining the metabolic effects of specific diets to enhance overall health and advance personalized health care. Recently, nutritional metabolomics research has focused on investigating metabolic pathways and biomarkers associated with nutrition and their interactions with various diseases, considering both individual and population levels. The goal is to pave the way for personalized health care in the future. Integrating metabolomic profiling with transcriptional and genomic analyses provides valuable insights into nutrient deficiency and supply mechanisms, highlighting their impact on cellular homeostasis during critical illness and recovery.

Given metabolism's central role in nutrition, metabolomics is emerging as a vital analytical tool in human nutritional research. As a result, nutritionists are increasingly incorporating metabolomics into their study designs. However, despite its growing significance, the potential of nutritional metabolomics, also known as nutrimetabolomics, to shape health policies has yet to be fully realized.[59,60] Achieving this requires collaboration within the research community to leverage the opportunities that nutritional metabolomics presents. The application of metabolomics across multiple fields of nutritional and food science research greatly enhances our understanding of chemical compounds in food. When combined with other omics technologies, metabolomics and its analytical tools play a crucial role in assessing diet-related health changes. This makes it an essential component in the development of evidence-based dietary guidelines.[54,57,61-63]

### 2.4. Microbiomics and PN

The large-scale dynamics of the microbiome can be analyzed using many of the same tools and principles applied in population ecology. Understanding the metagenome and its collective genetic information provides valuable insights into the functional properties of microbial communities. Both the microbiome and metagenome are likely to play significant roles in health and disease, making their study a key frontier in human genetics. Growing evidence highlights the impact of diet and other microenvironmental factors in shaping the composition and metabolic function of the human gut microbiota, which can significantly affect overall health.

Human endomicrobiota and gut microbiota profiles are of great interest in dietetic interventions for assessing the consequences of diet on gut microbiome diversity. Molecular technologies provide valuable insights into the complexity and diversity of gut microbial communities both within and across individuals. Dietary intake, especially macronutrients, plays a crucial role in shaping the composition and functions of these intricate populations. However, the effects of dietary fats and proteins on gut microbiota remain poorly defined. Short- and long-term dietary modifications can influence microbial profiles, and early-life nutrition can have lasting effects by modulating the immune system through microbial interactions. The influence of environmental factors, including lifestyle, on the gut microbiota is still not well understood.[50,64,65]

The primary goal is to customize nutritional interventions by enhancing the richness and diversity of the gut microbiota. Diet plays an essential role in shaping microbiota, serving both as an influencer and as a substrate. As food is processed by gut microbes, it generates small molecules that facilitate interactions between the host and microbiome. For example, microbiota-derived short-chain fatty acids are absorbed by the host, significantly contributing to overall nutrition.[66-71]

While long-term dietary habits influence the composition and activity of the trillions of microorganisms residing in the human gut, the speed and consistency of the gut microbiota's response to short-term macronutrient changes remain unclear.[72,73] Translating microbiota research into clinical applications for nutritional interventions presents challenges. However, advancements in analytical and computational approaches are helping to bridge these gaps. Integrating microbiota studies with other omics technologies, such as proteomics and metabolomics, enables the development of more precise functional profiles.[74-77] Furthermore, controlled studies are essential to identify diet-independent environmental factors that play a crucial role in shaping the gut microbiota ecosystem. Addressing these factors will open new avenues for designing personalized nutritional strategies, driving the advancement of PPM-guided nutrition.[78]

### 2.5. Interactomics

Proper nutrition plays a significant role in disease prevention, making nutritional interventions essential strategies within the framework of PPM. The emergence of nutrigenomics and nutriproteomics stems from the integration of nutrition, genomics, and proteomics, shaping the future of PPM-driven health care. In this sense, interactomics—a discipline at the intersection of bioinformatics and biology—focuses on studying molecular interactions within a cell, particularly between proteins and other molecules, as well as their functional effects. The goal of interactomics is to analyze and compare interaction networks (interactomes) both within and across species, identifying patterns of stability or change in these networks. From a computational biology perspective, an interactome network is modeled as a graph or system that maps key interactions essential for maintaining normal physiological functions in cells or organisms.[79-84]

Interactomics—as a rapidly advancing field within systems biology—and network biology are now positioned to intersect with PPM-guided personalized therapy. This integration combines traditional clinical records and non-invasive, advanced cardiac imaging tools with epigenetic information and deep learning techniques for comprehensive molecular phenotyping of CHD. This innovative approach holds the potential to discover new drugs from natural compounds, such as polyphenols and folic acid, and repurpose existing drugs such as statins and metformin. Several clinical trials have explored the use of interactomics-sensitive drugs in both primary and secondary prevention. Interactomics and network medicine apply network science methodologies to study disease pathogenesis. Various analytical techniques, including protein–protein interaction (PPI) networks, correlation-based networks, gene regulatory networks, and Bayesian networks, have been employed to identify and analyze key molecular networks involved in disease.

Proteins are crucial in most biological processes, and their interactions are essential for regulating biological functions. The development of large-scale PPI screening techniques, particularly high-throughput affinity capture coupled with mass spectrometry and yeast two-hybrid analysis, has generated vast amounts of PPI data and more complex, comprehensive interactomes.[85] Interactomics and network medicine leverage these integrated approaches to analyze big omics data—encompassing genetics, epigenetics, transcriptomics, metabolomics, and

proteomics—using computational biology tools. This combination has the potential to advance the diagnosis, prognosis, and treatment of complex diseases. However, several key challenges remain in interactomics and network medicine, including the incompleteness of the molecular interactome, difficulties in recognizing critical genes within genetic association regions, and the limited application of these approaches to human diseases.

## 3. Advances in food analytics and digitalization in PN and PF

Food analysis is an evolving field that focuses on the development of more robust, efficient, and sensitive analytical techniques. To achieve these goals, information technologies (IT) such as AI and ML, along with advanced computational resources, are employed to process and extract data (e.g., gathering dietary information) and to integrate model features for generating outputs that elucidate the complex relationship within large-scale "Big Data" datasets, which encompass numerous data points and variables (Figure 12).

Recent advances have led to significant developments in novel techniques in the following areas:

(i). Molecular methods and DNA-based techniques now enable faster and more precise detection of bacteria in foods, characterization of microbial communities, and identification of genetically engineered crops, all of which remain critical areas of investigation.

(ii). Biosensors are analytical devices composed of a specific biologically recognized element, such as enzymes, antibodies, or microbes, paired with a transducer that converts a biochemical response into an electrical signal. These devices are used to detect food components, including preservatives, colorants, and sweeteners, as well as contaminants such as toxins, pesticides, antibiotics, hormones, and microbes.

(iii). The development of advanced methodologies has led to the application of peptide nucleic acid-based technologies for food authentication and analysis, the refinement of immunoassay techniques to detect veterinary drug residues in food products, and the enhancement of methods for characterizing plant food allergens.[86] Figure 13 provides a comprehensive overview of the components and activities that constitute a fully integrated and PN service.

Figure 13 provides a comprehensive overview of the key elements and activities that constitute a PN-guided service. Specifically, it illustrates the process from utilizing various technologies for data collection, as previously discussed, to processing this information through big data analytics, algorithms, and AI to generate PN advice. In addition, it

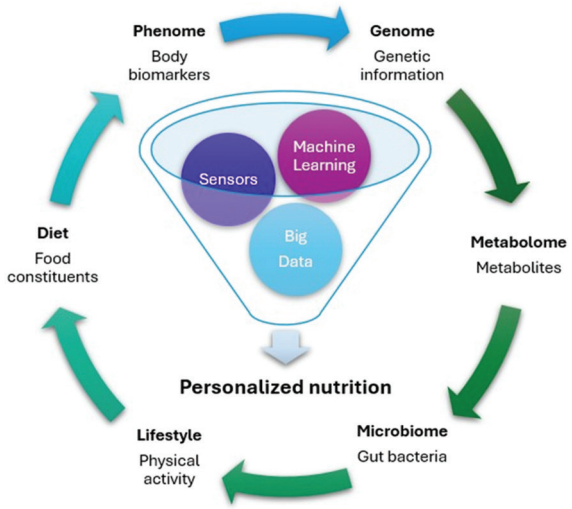


**Figure 12.** Big data analytics in personalized nutrition (PN) involves the genome, metabolome, microbiome, lifestyle, diet, and phenome. Nutrition plays a key role in our overall well-being, influencing both physical and mental health. As understanding of the intricate relationship between diet and health advances, PN has emerged as a promising strategy to optimize individual dietary choices. This approach involves customizing dietary recommendations based on unique characteristics such as genetics, metabolism, lifestyle, and health goals. By adjusting the diet to meet specific needs, PN can aid in managing chronic conditions, boosting the immune system, improving energy levels, and lowering the risk of diet-related diseases. Although the integration of digital technologies has facilitated technical advancements and the broader adoption of PN, challenges and ethical concerns remain, such as data privacy, algorithmic accuracy, and potential biases in data analysis. PN leverages the latest advancements in analytical instrumentation (e.g., omics) and computational tools (e.g., big data and AI) to gain deeper insights into the connections between foods, individuals, and health. This knowledge is then applied to the design of foods tailored to specific nutritional needs, ultimately promoting better health and well-being.[9]

highlights the importance of providing consumer feedback and establishing a continuous support system to monitor progress and encourage behavior changes that promote positive health outcomes. Meanwhile, digitalization can facilitate the adoption of PN and PF through the following means:

(i). Data collection and analysis: Digital tools and platforms allow users to gather and track their health-related data, such as blood sugar levels, cholesterol, lipid profiles, and nutritional and dietary biomarkers, through wearables, mobile applications, and genetic testing. Advancements in profiling algorithms enable the rapid management of vast amounts of data and the identification of patterns and correlations.

(ii). Personalized meal planning: Digital platforms can generate and recommend customized meal plans based on an individual's dietary preferences, genetics, allergies, nutritional requirements, and health

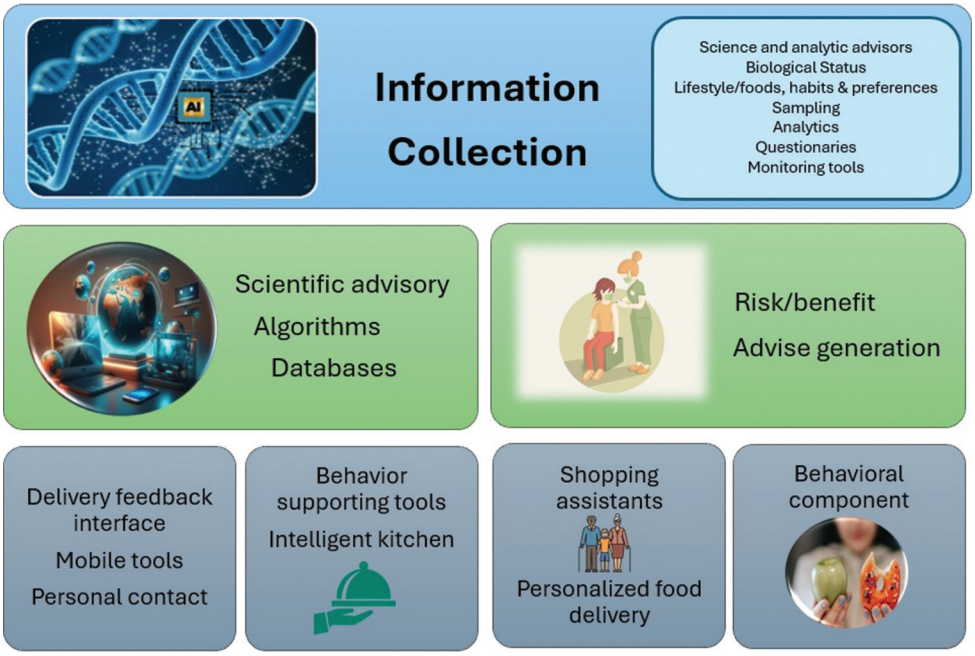


**Figure 13.** Overview of the elements and activities that constitute a fully integrated personalized nutrition (PN) service. PN is rapidly gaining traction as an innovative approach to guiding individuals toward informed dietary choices. It is built on two fundamental pillars: (i) The scientific, analytical, and technological components that ensure the accuracy and effectiveness of nutritional recommendations, and (ii) the equally vital behavioral dimension that ensures these recommendations are practical and actionable. While the growing interest in PN aligns with strong societal trends, delivering such a service will require innovative strategies beyond those currently employed by traditional nutrition and health-care sector stakeholders. It is likely that a new ecosystem of interconnected companies will emerge to meet this evolving demand. By shaping food choices, PN has the potential to exert a profound influence on the food supply chain and on the way information about ingredients and products is communicated to consumers.[87]

objectives. These plans may include detailed recipes and portion sizes tailored to the individual.

(iii). Behavioral tracking: PN tools assist users in monitoring their progress toward defined health goals, providing feedback and recommendations to enhance motivation and adherence.

(iv). Remote coaching and support: Digitalization enables individuals to interact remotely with nutritionists, dietitians, and other health professionals. Through video consultations and messaging, individuals can obtain PN advice and support without the need for in-person visits, making the overall process more convenient.

(v). Integration with e-commerce: Digital platforms can connect health-conscious users with PN products and supplements available on e-commerce sites, making it more convenient to follow their customized dietary plans.

Digitalization plays a significant role in addressing challenges by leveraging technology to analyze health data, disseminating knowledge and awareness related to PN and PF, and facilitating remote consultations with nutrition experts. As digitalization stakeholders continue to expand into this domain, the PN market is expected to experience further disruptions and cross-industry collaborations (Figure 14).

As shown in Figure 14, the potential market for PN is huge. Most commercial PN-driven interventions are delivered directly to consumers by online platforms. The public shows a strong interest in receiving PN guidance; however, concerns regarding the reliability of certain primary care service providers, the credibility of information sources, and the security of personal data remain. Other influencing factors include individuals' preferences for primary care services and their diverse socioeconomic backgrounds. In this regard, direct-to-consumer DNA testing serves as an example of primary care services available to the public.[89-91] In this context, guiding principles should be applied when designing and implementing PN research, considering its multidisciplinary nature.[92] Designers, researchers, and dietitians should collaborate to conduct a thorough review of PN research, utilizing a standardized definition to guide the development of future NP-driven practices. This effort should also address challenges arising from rapid technological advancement and the lack of consensus on integrating these tools into clinical settings. Establishing stakeholder consensus on standardized methods for implementing multiomics technologies and IT-assisted support could accelerate their adoption in clinical practice, enhancing NP-based recommendations for disease prevention and treatment. When vital research

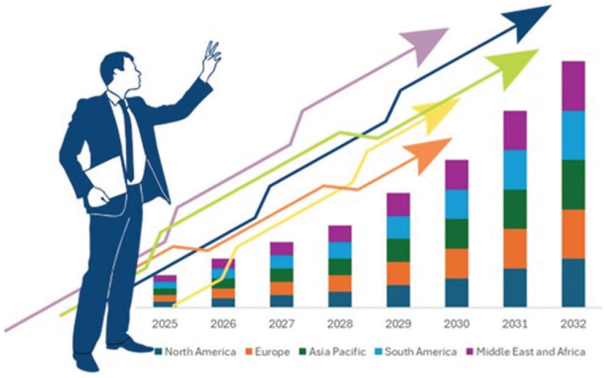


**Figure 14.** Global personalized nutrition (PN) market. The global PN market is expected to reach USD 20.14 billion by 2029. Dietitians worldwide have been incorporating PN into their practice since the concept was first introduced. This approach delivers tailored nutritional guidance based on an individual's physical, emotional, and clinical needs, considering factors such as genetic makeup, physical activity, microbiome composition, eating habits, and sleep patterns. The PN market is segmented by product type, age group, suppliers, dosage forms, end-users, and applications. Analyzing growth trends within these segments provides valuable market insights, helping identify key opportunities and support strategic decision-making. The PN market spans multiple regions, including North America (e.g., United States, Canada, and Mexico), Europe (e.g., Germany, Sweden, Poland, Denmark, Italy, the United Kingdom, France, Spain, the Netherlands, Belgium, Switzerland, Turkey, and Russia), Asia-Pacific (e.g., Japan, China, India, South Korea, Australia, New Zealand, Vietnam, Singapore, Malaysia, Thailand, Indonesia, and the Philippines), South America (e.g., Brazil and Argentina), and the Middle East and Africa (e.g., United Arab Emirates, Saudi Arabia, Oman, Qatar, Kuwait, and South Africa). North America leads the PN market, driven by growing consumer awareness of health and wellness. The Asia-Pacific region is projected to experience significant growth between 2022 and 2029, largely due to rising obesity rates and increasing demand for customized health solutions.[88]

and reviews are unified, progress can be achieved in the field of human health for NPs.

Diet is more than just the sum of its individual components, and food is not merely a collection of nutrients. Instead, the chemical, physical, and biological properties contribute to sensory, safety, and functional attributes that extend beyond basic nutrition. Foods with similar ingredients but different structures—such as pre-cooked versus cooked rice with distinct starch compositions or raw versus homogenized milk with varying fat globule sizes—exhibit differences in digestion, absorption, and metabolic responses. PF is an emerging discipline within food and nutrition that integrates high-throughput omics technologies to enhance human health, well-being, and nutritional knowledge. This field encompasses a wide range of research areas, including nutrigenomics, and aims to explore the mechanisms of bioactive food components in the body, quantify dietary biomarkers to assess health states, evaluate food quality and safety, and analyze the body's biological response to different dietary patterns.

Foodomics is recognized as a subdiscipline within the four major branches of omics, including genomics, proteomics, transcriptomics, and metabolomics. The emergence of genomics and proteomics in the 1990s was driven by the advent of high-throughput technologies for rapid DNA sequencing and mass spectrometry (MS)-based protein identification.[93] This technological progress led to the development of microarrays, enabling the rapid and comprehensive analysis of gene expression and giving rise to transcriptomics in the early 2000s. These advancements, in turn, facilitated the emergence of metabolomics.

Metabolomics employs high-throughput analytical chemistry techniques—such as liquid chromatography-MS, nuclear magnetic resonance, and gas chromatography-MS—to characterize the metabolome, the collection of small molecules involved in metabolism.[94] Although metabolomics is not as rapid or high-throughput as genomics or proteomics, it allows researchers to analyze hundreds or even thousands of metabolites simultaneously, rather than focusing on one or a few compounds.[86] This capability has led to several large-scale metabolomic projects aimed at identifying and mapping the metabolomes of microbes, plants, and humans. These studies typically employ liquid chromatography-MS, nuclear magnetic resonance, and gas chromatography-MS, or a combination of these techniques to quantify and profile metabolites in cells, tissues, and biofluids. In addition, more targeted metabolomic research has been conducted to explore the metabolic responses of humans to various foods and dietary components, such as soy, citrus fruits, nuts, meats, and tea, further advancing our understanding of the complex interactions between diet and metabolism.[95]

Metabolite levels are biologically significant because, similar to the canary in a coal mine, they are often the first to respond to both internal physiological changes and external environmental factors. This makes metabolites valuable for various purposes, including monitoring the body's immediate reactions to stimuli, developing biomarkers for early disease detection, and enhancing food safety. While there is clinical evidence supporting the establishment of a comprehensive and integrated framework for PPM-based nutritional interventions in clinical settings, this evidence is still limited. For example, interventional nutrition has enabled the implementation of specific diets that have saved patients and their families from severe outcomes. Genetic testing has helped to identify patients who are slow or fast metabolizers in the context of wellness. While genomics-based PN has been applied, PPM-based nutrition still faces challenges due to the lack of sufficient diagnostic tests for full integration into clinical practice. To be fully effective,

PPM-based nutrition should be integrated into the daily diet to prevent and mitigate diseases commonly observed in metabolic disorders.

Digitalization has transformed PN and PF by empowering people to assume responsibility for their own health, access tailored guidance, and make informed choices for improved well-being. Digital tools can continually improve data accuracy, providing users with more reliable and valuable insights for making informed dietary choices. Technological advancements, particularly in multiomics and IT resources, have significantly propelled the field of PN. These innovations hold great promise in developing objective biomarkers for specific foods—through bioactive components—and nutrient intake, providing an effective complement to current self-reported dietary recall methods. Proper application of these guiding principles could pave the way for a standardized definition of PN and facilitate a consensus on how to implement these tools in clinical settings.

Nutrition experts and dietitians must not only grasp the fundamentals of these advancements but also stay informed about ongoing research to position themselves as leaders in delivering PN-guided therapies. Achieving this requires collaboration among a diverse group of professionals, including biodesigners, translational and clinical researchers, nutritionists, clinicians, nurses, bioinformaticians, statisticians, chemists, and other key stakeholders. The new frontier in nutritional sciences lies in our ability to predictably engineer physiological networks to optimize diet, health, and disease management. This multidisciplinary integration is essential for developing the knowledge needed to establish evidence-based PPM-based nutrition. Ultimately, this collaboration will enable more precise dietary interventions and improve health monitoring strategies.[96]

## 4. The future of PN in PPM

PN, an interdisciplinary field, examines how nutrients interact with the body to support health and well-being. In daily practice, PN provides tailored health advice regarding food intake and health goals, such as the guidance traditionally offered by doctors, dietitians, and nutritionists (Figure 15).

Nutrition is inherently complex, influenced by a wide range of internal and external factors. To fully understand and address this complexity, holistic and network-based approaches are needed to uncover the dynamic interactions within these systems across both temporal and spatial scales. Hence, the concept of PPM-based nutrition is to deliver precise nutritional recommendations personalized to each individual to promote a healthier lifestyle. An individual's

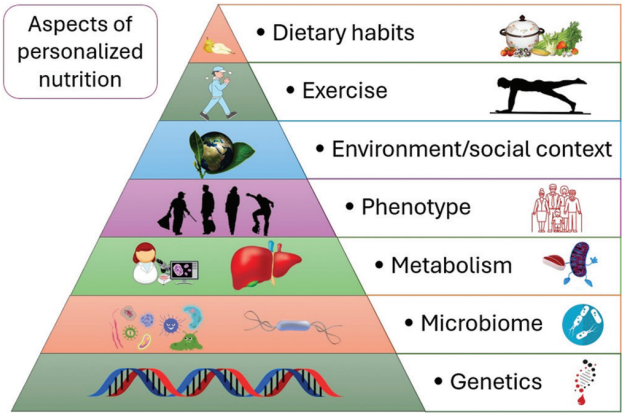


**Figure 15.** Personal input data elements of personalized nutrition (PN). In addition to phenotype data collected through questionnaires, tracking devices, and software applications, there is an increasing emphasis among providers on incorporating personal genetic or genomic information, including the genetic makeup of the gut microbiome. Efforts are currently underway to establish an internationally agreed-upon framework that defines the role of these scientific methods within PN. While a precise and uniform definition of PN is still lacking, the inclusion of genetic variants in personalized dietary recommendations is gaining support, despite limited scientific evidence supporting gene-based dietary guidance. This article aims to provide a science-based perspective on gene-based PN and weight management. Most studies conducted to date have found little or no clinical evidence supporting the effectiveness of gene-based PN. At present, gene-based nutrition is not applicable to the treatment of obesity. Nevertheless, personalized dietary recommendations based on an individual's genetic profile hold promise as an innovative approach for the prevention and treatment of obesity. Future human intervention studies are needed to establish the clinical validity of gene-based dietary recommendations.[97]

integrative nutritional biomarker profile, combined with the characterization of specific food components, can determine their optimal PPM-based nutrition. Such advancements are driving the development of innovative strategies for managing chronic diseases. PPM-based nutrition holds significant potential for promoting health by incorporating comprehensive nutrigenomics analyses while accounting for an individual's genetic profile. Consequently, there is a growing need to identify novel nutritional biomarkers or biomarker patterns that connect diet with health, thereby enhancing our understanding of the role of nutrition in both health and disease.[98-100]

The foundation of PN is supported by several factors, including advancements in food analytics, the growing prevalence of nutrition-related diseases, the expansion of public health programs, and the increasing role of bioinformatics and mathematical modeling in nutrition science. In addition, the concept of gene–diet interaction and rising consumer demand for healthier food choices further drive this field forward. The rapid progress in omics technologies and related analytical techniques has significantly expanded their application in nutrition

science. By generating high-throughput molecular data, omics enables a deeper understanding of the complexities of nutrition, fundamentally reshaping the field.

From both translational and clinical perspectives, staying informed about these emerging directions in nutritional science is essential for advancing health and disease management. By collecting individual data at the genetic, phenotypic, medical, and nutritional levels and using these data to provide personalized dietary recommendations, PN aims to maintain or improve health. Disease prevention and the treatment of existing conditions further enhance its effectiveness. Enhancing human health through PN has become a subject of significant interest. In this sense, multiomics can be leveraged to analyze the microbiota, metagenome, proteome, transcriptome, and metabolome, offering a detailed understanding of an individual's physiological state. As PN gains momentum, advancements in multiomics technologies are expected to shape future practice by facilitating the identification of objective nutritional biomarkers that link dietary intake with health status.[86]

The progress of PN will be driven by several key factors linked to the growing body of scientific evidence. First, establishing a robust theoretical framework that identifies the most relevant individual characteristics for PN is essential. Second, well-structured intervention studies must provide evidence of PN's effectiveness and cost-efficiency. Finally, implementing a regulatory framework will be crucial for safeguarding public interest and instilling confidence among health-care professionals and policymakers. Achieving these goals will require a significant expansion of scientific research in the field.[95]

In this new dietary era, marked by a societal shift from merely consuming what is available to making healthy and conscious decisions, rethinking and refining dietary habits has become increasingly important as we move toward PN tailored to individual needs. In addition, by leveraging advanced analytics, PN can deliver personalized dietary advice that accounts for each individual's unique characteristics, preferences, and health goals. This approach offers the potential to enhance overall health, support chronic disease management, and maximize athletic performance. As technology and research continue to advance, PN provides a new avenue for improving individual health and well-being.

The integration of PN with PF is evolving beyond a mere trend, driven by a new generation of consumers seeking clarity amid the confusion of mass-marketed, one-size-fits-all nutritional products. The fusion of technological advancements and growing consumer awareness of nutrition and wellness—along with greater access to relevant information—is driving the development of innovative health products and services tailored to individual needs. By leveraging online tools such as questionnaires, along with advanced data sources such as wearable devices, DNA analysis, blood biomarkers, and microbiome profiling, PN-driven approaches are advancing. These strategies enable the creation of products that are more precisely formulated to align with an individual's lifestyle, genomic predisposition, and metabolic requirements, surpassing anything currently available.

### 4.1. Food products enriched with autoprobiotics

It has been shown for the first time that exposure to probiotic preparations under conditions simulating space flight factors inherent in interplanetary expeditions (e.g., hypomagnetic environment, altered radiation background) does not lead to changes in their quality. Therefore, probiotics may be used in expeditions to the Moon, following recommendations established under terrestrial conditions. The use of food products enriched with autoprobiotics exerts an effective stabilizing effect on the human intestinal microbiota in experiments simulating the impact of space flight factors. Thus, food products enriched with autoprobiotics are considered promising for medical support during long-term, including interplanetary space flights. This work experimentally substantiates the development of food products incorporating autologous microorganisms, representatives of the protective intestinal microflora. The impact of a combination of altered environmental factors (e.g., radiation exposure, freezing, hypomagnetic environment) does not adversely affect the probiotic properties of individual cultures or their associations. These findings suggest that technologies for enriching food products with autoprobiotics may be applicable to future programs for deep space exploration.[101]

## 5. Conclusion

Human PN is a specialized branch of PPM that focuses on the biochemical connections between food and the human body. The shift from a healthy state to disease is influenced by variations in gene and protein expression, leading to the emergence of "omics sciences" as a critical field of study.

As discussed in the previous sections, significant progress has been made in PN due to a growing body of research supporting its effectiveness. Nonetheless, a deeper understanding of the complex interactions between genes and diet remains essential, especially as the development of novel food products further increases complexity.

The successful implementation of PN models in real-world settings is crucial for transforming theoretical concepts into practical applications. Challenges such as

ethical considerations surrounding genomic data sharing, the high variability of multiomics data in biological samples, and the shortage of skilled professionals in big data generation, analysis, and management must be addressed to ensure the continued advancement of PN.

With advancements in omics techniques, the concept of food has evolved beyond being merely a source of energy, macronutrients, and micronutrients. It is now recognized as a key determinant of overall health and well-being.

The intricate relationship between micronutrients and gene expression plays a crucial role in various pathophysiological processes and offers valuable insights into disease prevention. Understanding these interactions can help delay the onset of chronic disorders, paving the way for more targeted and effective nutritional strategies.

Emerging technologies in omics-guided applications, such as nutrigenomics and deep phenotyping, have enabled the collection of extensive data on genetic markers, clinical indicators, precise body composition metrics, and dietary consumption. The complex interplay between genetics, dietary habits, and lifestyle influences an individual's risk of developing CVD. For instance, in cases of hypercholesterolemia, a key challenge lies in the misalignment between genes regulating lipid metabolism and the modern diet, compounded by various lifestyle factors. The primary challenge in both PN and PPM is effectively translating these insights into clinically actionable and relevant recommendations for improved health outcomes.

A deeper understanding of nutrient–gene–metabolite pathways will enable a more integrated approach to cellular studies at different levels. In this context, the interactions between gut microbiota and food should be thoroughly assessed. PN should focus on selecting the most appropriate foods for each individual, based on their effects on gene expression and gut microbiota composition. In addition to their primary function as an energy source, foods will increasingly be chosen for their bioactive components.

PN has the potential to revolutionize health-care by offering tailored meal plans and progress tracking, designed to align with an individual's unique characteristics and health goals. To fully grasp the integrated relationship between PN and health, it is essential to understand the underlying mechanisms governing systemic flexibility. Many of these mechanisms are influenced by PN, directly impacting health outcomes and overall well-being.

Following PN guidelines can significantly enhance disease management by focusing on diet-based biochemistry, insulin regulation, nutrient absorption, and nutritional tracking, ultimately leading to better health outcomes. The role of nutrients in regulating gene activity, both directly and indirectly, opens up new pathways for PN in the prevention and treatment of chronic health conditions.

We are entering an era defined by PN, PF, and molecular food design, which is reshaping our approach to health and wellness. This shift calls for the development of global scientific, clinical, social, and educational initiatives focused on PPM to foster this emerging branch. As we look to the future, digital advancements will propel PN-based therapies to new levels of accessibility, personalization, and precision. Technologies such as ML and big data analytics are set to revolutionize the medical nutrition sector, while their integration into wearable devices, mobile platforms, and smart devices will further enhance accessibility for patients.

In the coming years, the integration of PF, PN, systems biology, and general pathology will yield valuable insights into areas such as host–microbiome interactions, nutritional immunology, pathogen resistance in food microorganisms, and farm–animal production. This unified approach will also help us better understand post-harvest phenomena by linking genetic and environmental responses, ultimately identifying key bionetworks that influence health outcomes.

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ORIGINAL RESEARCH ARTICLE

# Leveraging convolutional neural networks to address overfitting and generalizability in automated bone fracture detection

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## Abstract

Bone fractures represent a significant health burden that demands precise and timely diagnosis to optimize patient outcomes. To address challenges such as data scarcity, overfitting, and generalizability, this study investigates the use of convolutional neural networks (CNNs) for automated fracture detection in X-ray images. A dataset of 4,900 X-ray images was preprocessed and evenly divided into training, validation, and test subsets. The proposed CNN model directly addressed generalizability and overfitting issues by prioritizing training stability and incorporating advanced techniques. These techniques included batch normalization and dropout to enhance stability and mitigate overfitting, with five-fold cross-validation yielding an average accuracy of 95%. Validation and held-out test datasets achieved accuracies of 95.8% and 94.5%, respectively, while external validation on an independent dataset confirmed the model's generalizability at 91.7%. High recall rates across all datasets underscore the model's capacity to minimize missed fracture diagnoses, whereas slightly lower precision on external data indicates a need to address false positives. These findings suggest that artificial intelligence is best deployed as a screening tool, serving as an initial triage mechanism that flags potential cases for further human-guided evaluation, thereby enhancing clinical efficiency without replacing the diagnostic expertise of healthcare professionals.

***Keywords:*** Bone fracture detection; Artificial intelligence; Convolutional neural networks; Medical imaging; Deep learning

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## 1. Introduction

Bone fractures are a common and significant health concern, often requiring prompt and accurate diagnosis for effective treatment. In recent years, the integration of artificial intelligence (AI) and machine learning (ML) into radiology has enhanced the field of medical imaging, particularly in the domain of bone fracture detection.[1] This study presents an approach to automated bone fracture detection using convolutional neural networks (CNNs), addressing key challenges in the field and contributing to the advancement of diagnostic accuracy and efficiency in orthopedic imaging.

The application of AI in radiology has seen remarkable progress, with AI-based tools being used to enhance the accuracy and efficiency of diagnosing bone fractures.[1] These tools are designed to assist radiologists by providing faster and more consistent fracture identification, which is crucial for timely and effective treatment.[1,2] Recent studies have demonstrated the capability of AI algorithms to accurately detect and classify fractures, especially in the wrist and long bones, using X-ray images.[3]

CNNs have emerged as a cornerstone in medical imaging analysis, particularly in orthopedics, due to their ability to process and analyze complex image data with high accuracy.[4] These networks are structured to mimic the human visual cortex,[5] allowing them to identify patterns and features in medical images that may be difficult for human observers to discern.[6,7] In the context of bone fracture detection, CNNs have shown promising results, with some studies indicating that AI is noninferior to clinicians in terms of diagnostic performance.[8]

### 1.1. Common challenges

Despite the advancements in AI-based fracture detection, several challenges persist in the field.[9] High-quality, annotated datasets are essential for training effective AI models. However, there is often a scarcity of such datasets, which can limit the performance and generalizability of fracture detection models. AI models, particularly deep learning models, often overfit to the training data, especially when the dataset is small or lacks diversity.[10,11] This limits the model's ability to generalize to new, unseen data.

A persistent challenge in AI-assisted fracture detection lies not in achieving high nominal accuracy but in ensuring that such metrics stem from rigorously validated models capable of real-world generalization.[1,12,13] Many studies report exceptional performance, yet methodological shortcomings, such as inadequate data splitting, insufficient validation protocols (systematic procedures for evaluating model performance, including partitioning data into training, validation, and test sets to prevent overfitting), or reliance on homogeneous datasets, often inflate internal benchmarks at the expense of clinical applicability.[14-17] This discrepancy highlights a critical disconnect; models optimized for accuracy on internal data may fail catastrophically when confronted with external populations or operational heterogeneity, a limitation amplified by inconsistent validation practices across the field.[9,18] The goal of our study is to directly address this gap by prioritizing training stability and generalizability over raw performance through methodological rigor in data handling and model validation.

Proper data splitting is essential to developing models that generalize well to unseen data. This involves partitioning datasets into different subsets, such as training, validation, and test sets.[14-19] The graph in Figure 1 illustrates the number of studies published each year using two-way and three-way data splitting strategies from 2007 to 2022. It highlights a significant shift in the research community's approach to data splitting in ML studies. In the earlier years, particularly from 2007 to 2017, most studies employed two-way splitting, where the dataset is divided into a training set and a testing set. This method lacks a validation set, which is essential for tuning hyperparameters and preventing overfitting. Without a validation set, models may not generalize well to unseen data, leading to inefficient ML training and distorted results. This limits the model's ability to generalize to new, unseen data. Particular attention must be given to avoid data leakage, where information from the test set inadvertently influences model training, leading to inflated and unreliable performance metrics.

Starting around 2018, the graph shows several studies adopting three-way splitting. This approach involves splitting the data into three sets: training, validation, and testing. The validation set is used during model development to fine-tune hyperparameters and select the best model before final evaluation on the test set. By 2022, the number of studies using three-way splitting surpasses those using two-way splitting, indicating a positive trend toward more robust ML practices.

The increasing adoption of three-way splitting reflects a growing awareness of the pitfalls of overfitting and the importance of model validation. Without a validation set, there is a risk of inadvertently tuning the model to perform well on the test set, which can lead to overly optimistic performance estimates and poor generalization.[17-20] When

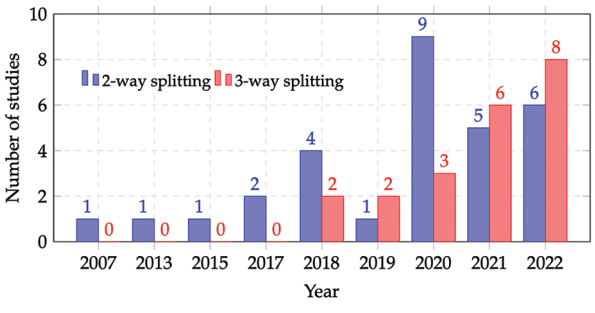


**Figure 1.** Yearly trend in the number of studies employing two-way and three-way data splitting strategies in artificial intelligence-assisted bone fracture detection research (2007–2022). The graph highlights the increasing adoption of three-way splitting, reflecting improved validation practices and model generalizability in machine learning. Data derived from Jung *et al.*[9]

studies do not demonstrate that their models are properly converged and well validated, it becomes difficult to trust their reported metrics. This is because overfitting may occur, where the model fits too closely to the training data and fails to generalize to new, unseen data. Without robust validation practices, such as using a separate validation set to monitor model performance during training, overfitting can remain undetected. Consequently, the reported high accuracy may not reflect the model's true performance in real-world applications, undermining the reliability of the study's findings.[19-21] Therefore, it is essential for studies to adopt proper validation strategies to ensure that their models generalize well and that their reported metrics are trustworthy.

Furthermore, many AI models, especially deep learning models, are often considered "black boxes," making it difficult to interpret their decision-making process. This lack of transparency can hinder clinical adoption.[22-24] Integrating AI tools into existing clinical workflows can be challenging, requiring changes in how radiologists and other healthcare professionals operate. There is a need for standardized performance metrics to evaluate and compare different AI models effectively. Current studies often use varied metrics, making it difficult to assess their relative performance.[13,25] Table 1 summarizes the key challenges in automated bone fracture detection and the corresponding solutions implemented in this study to address these issues.

By addressing critical aspects of automated bone fracture detection, this study provides insights into the development of reliable AI systems for medical imaging. It introduces an ML model that demonstrates strong performance and highlights its potential for clinical application through rigorous validation and an emphasis on generalizability.

The subsequent sections provide a detailed account of the methodology, followed by a presentation of the results, and a discussion on the implications of the findings for the future of AI-assisted bone fracture detection in clinical practice. This research represents an important advancement in improving the accuracy and efficiency of orthopedic imaging diagnostics, with the ultimate goal of enhancing patient care and outcomes.

### 1.2. Aims of the study

The primary aim of this study is to develop and validate a CNN model for automated bone fracture detection in X-ray images that specifically addresses the challenges of overfitting and limited generalizability commonly observed in medical imaging AI applications. Through methodological rigor in data handling and validation protocols, this study seeks to create a robust model that maintains high diagnostic performance when applied to diverse clinical scenarios and external datasets.

**Table 1. Summary of challenges in automated bone fracture detection and the corresponding solutions implemented in this study**

| Challenge | How it is addressed |
|---|---|
| Limited annotated data in the field | Curated a comprehensive dataset of 4,900 X-ray images, carefully preprocessed and standardized to ensure high-quality input for the model. |
| Overfitting and suboptimal CNN architecture design | Developed an advanced CNN architecture with convolutional layers for feature extraction, batch normalization, rectified linear unit activations, and dropout layers to mitigate overfitting. |
| Skewed dataset splits affecting reliability | Employed *k*-fold cross-validation to evaluate model performance across multiple data splits, ensuring reliable and robust performance metrics. |
| Lack of generalizability to unseen data | Performed external validation on an independent dataset, confirming the model's high accuracy and applicability in diverse clinical settings. |
| Ensuring high performance | Achieved high validation accuracy and test accuracy, confirmed with testing on external data, along with high sensitivity and specificity, showcasing strong reliability for clinical use. |
| Integration into clinical workflows | Highlighted the importance of clinical integration, with future work focusing on assessing the system's impact on diagnostic accuracy and efficiency in real-world settings. |

Note: The table highlights the limitations addressed, including dataset preparation, overfitting, generalizability, and methods to ensure high performance and clinical integration.
Abbreviation: CNN: Convoluted neural network.

The secondary aims of this study are:

(i) To implement and evaluate a validation strategy including three-way data splitting and *k*-fold cross-validation to ensure proper model training and assessment
(ii) To quantify the model's performance across internal validation, test datasets, and external datasets using multiple metrics (accuracy, precision, recall, and F1-score) to provide a complete picture of its clinical utility
(iii) To assess the model's generalizability by comparing its performance on an external dataset with its performance on internal datasets, thereby evaluating its potential for real-world clinical application
(iv) To analyze error patterns and identify specific challenges in fracture detection that may impact the model's performance when applied in diverse clinical settings.

## 2. Materials and methods

Datasets were sourced from publicly available Kaggle repositories containing several types of fractures, pre-classified as either fractures or non-fractures.[26,27] Database 1 provided a curated dataset of X-ray images,[26] while Database 2 offered additional annotated data for further testing.[27] Since both datasets are open-access and have been thoroughly de-identified, the potential risks associated with privacy breaches, re-identification, and misuse of sensitive information are effectively eliminated. Both datasets were preprocessed to ensure consistency as follows.

### 2.1. Dataset preparation

The dataset used in this study consisted of 4,900 X-ray images organized into two classes: fractured and not fractured. To ensure consistency, all images were resized to 224 × 224 pixels, and grayscale images were converted to the red, green, blue (RGB) format to match the input requirements of the CNN. This preprocessing step can be represented as Equation I:

$$I_{RGB} = Resize\ (I_{input}, 224\times224) \quad \text{(I)}$$

where $I_{RGB}$ represents the preprocessed RGB image, and $I_{input}$ is the original image.

The dataset was divided into three subsets: 70% (3,430 images) for training, 15% (735 images) for validation, and 15% (735 images) for testing. The distributions were checked to ensure balanced representation of the target classes across all subsets.

The preprocessing pipeline prioritized compatibility with established CNN architectures while aligning with clinical imaging standards. Resizing images to 224 × 224 pixels balanced computational efficiency with preservation of fracture-relevant anatomical detail, ensuring features like cortical discontinuities remained resolvable. Grayscale conversion to RGB accommodated pretrained models without altering diagnostic content, as fracture detection primarily relies on structural contrasts rather than spectral depth. Class-balanced splitting across training, validation, and testing subsets mitigated biases in fracture prevalence, ensuring model evaluations reflected real-world diagnostic challenges rather than dataset-specific artifactual advantages.

### 2.2. Model architecture

The CNN architecture used in this study comprised a series of convolutional layers, batch normalization layers, rectified linear unit activation functions, max-pooling layers, dropout layers, and a fully connected layer for final classification. The architecture is summarized as follows:

(i) Input layer: Accepts images of size 224 × 224 × 3
(ii) Convolutional layers: Extract spatial features using filters of size 3 × 3
(iii) Batch normalization: Normalizes activations to improve training stability
(iv) Rectified linear unit activation: Applies the activation function $f(x) = \max(0,x)$, to introduce non-linearity
(v) Max-pooling layers: Reduce spatial dimensions using pooling windows of size 2 × 2
(vi) Dropout layers: Introduce a dropout rate of 20% to mitigate overfitting
(vii) Fully connected layer: Maps feature representations to the two output classes
(viii) Softmax layer: Converts outputs to probabilities for classification:

$$P\left(y=i|x\right)=\frac{\exp\left(z_i\right)}{\sum_{j=1}^{k}\exp\left(z_j\right)} \quad \text{(II)}$$

In Equation II, $z_i$ represents the logit for class $i$, and $k$ is the number of classes (in this case, $k = 2$).

The architecture strategically interleaves feature extraction and regularization layers to optimize fracture pattern recognition while curbing overfitting. Convolutional layers progressively localized multiscale fracture signatures, from pixel-level intensity gradients to macro-scale trabecular disruptions. Batch normalization stabilized activations across variations in X-ray contrast, a common source of domain shift. Dropout layers explicitly disrupted co-adapted feature reliance during training, forcing the network to consolidate robust diagnostic cues resilient to missing inputs.

### 2.3. Training procedure

The model was trained using the Adam optimizer with an initial learning rate of 0.001. The mini-batch size was set to 32, and training was conducted over 10 epochs. The training loss $L$ was computed using the categorical cross-entropy loss function (Equation III):

$$\mathcal{L}=-\frac{1}{N}\sum_{i=1}^{N}\ \sum_{j=1}^{k}y_{ij}\log\hat{y}_{ij} \quad \text{(III)}$$

where $y_{i,j}$ is the true label, $\hat{y}_{ij}$ is the predicted probability for class $j$, and $N$ is the total number of samples in the mini-batch.

Validation performance was monitored at regular intervals to ensure effective learning. The dropout layers were only applied during the training phase, ensuring that validation metrics reflected true model performance. The training regimen balanced convergence speed with

generalization capacity. The Adam optimizer's adaptive learning rates mitigated gradient instability during early training phases, while the conservative initial learning rate ensured fine-grained parameter updates critical for distinguishing subtle fracture phenotypes. Restricting training to 10 epochs prevented over-optimization to transient batch-level noise, as evidenced by stabilized validation loss trajectories. By applying dropout exclusively during training, and disabling it during validation, the metrics accurately reflected the model's inherent diagnostic capability rather than transient regularization effects.

### 2.4. *k*-fold cross-validation

To assess the model's generalizability, a *k*-fold cross-validation strategy was employed with $k = 5$. The dataset was split into *k* partitions, and the model was trained and validated on *k−1* folds while testing on the others. The process was repeated for all folds, and the cross-validation accuracy was computed as Equation IV:

$$CV\ accuracy = \frac{1}{k}\sum_{i=1}^{k} accuracy_i \quad \text{(IV)}$$

where $accuracy_i$ is the validation accuracy for fold *i*.

The *k*-fold approach probed model stability under variations in data composition. By cyclically excluding distinct patient subgroups during training, the method simulated multicenter validation scenarios and quantified performance variance attributable to sampling biases. Repeated retraining across folds ensured architectural decisions generalized beyond feature distributions in individual splits. This process mirrored clinical reality, where AI tools must maintain diagnostic fidelity across heterogeneous patient populations and acquisition protocols.

### 2.5. Algorithm pseudo-code

The training procedure, including cross-validation, is summarized in Algorithm 1.

**Algorithm 1.** Training and cross-validation procedure

**Input**: Dataset *D*, number of folds *k*, number of epochs *E*, mini-batch size *B*
Split *D* into *k* folds
**for** $i = 1$ to *k* **do**
Assign *i*-th fold as the validation set $D_{val}$, remaining folds as training set $D_{train}$
Initialize CNN model parameters
**for** *epoch* = 1 to *E* **do**
Divide $D_{train}$ into mini-batches of size *B*
**for** each mini-batch (**X**, **y**) **do**
Perform forward pass to compute predictions $\hat{\mathbf{y}}$
Compute loss *L* (Eq. 4)
Backpropagate gradients and update parameters using Adam
**end** for
Evaluate model on $D_{val}$
**end** for
Compute validation accuracy for fold *i*
**end** for
Compute cross-validation accuracy (Eq. 5)
**Output**: Trained model, cross-validation accuracy

### 2.6. Evaluation and testing

The final model was evaluated on the test dataset and an external dataset to assess generalizability. Performance metrics, including accuracy, sensitivity, specificity, and confusion matrices, were computed. The accuracy was calculated using a formula that accounts for the binary classification nature of the problem. Specifically, accuracy was defined as the ratio of correctly classified samples to the total number of samples, incorporating true positives (TPs), true negatives (TNs), false positives (FPs), and false negatives (FNs). This is expressed mathematically as Equation V:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \quad \text{(V)}$$

In this context, TP represents the number of fractured cases correctly identified as fractured, while TN denotes the number of non-fractured cases correctly identified as non-fractured. FP corresponds to non-fractured cases incorrectly classified as fractured, and FN represents fractured cases incorrectly classified as non-fractured. This formulation provides a comprehensive measure of the model's performance by considering all possible outcomes in the classification process.

To further evaluate the model's performance, additional metrics were computed. Precision, which measures the proportion of correctly identified positive cases out of all predicted positive cases, was calculated using Equation VI:

$$Precision = \frac{TP}{TP + FP} \quad \text{(VI)}$$

Recall, also referred to as sensitivity or the TP rate, was used to assess the model's ability to identify all actual positive cases. It is defined as Equation VII:

$$Recall = \frac{TP}{TP + FN} \quad \text{(VII)}$$

To balance precision and recall, the F1-score was computed as the harmonic mean of these two metrics, providing a single measure that accounts for both FPs and FNs. The F1-score is given by Equation VIII:

$$F1-score=2\times\frac{Precision\times Recall}{Precision+Recall} \quad \text{(VIII)}$$

These metrics are particularly useful in evaluating the model's performance on imbalanced datasets, where one class (e.g., fractured cases) might be underrepresented. By combining these measures, the evaluation provides a detailed understanding of the model's strengths and weaknesses. In addition, confusion matrices are generated to visualize the distribution of predictions across the TP, TN, FP, and FN categories. This analysis offers further insights into the model's performance and its potential for deployment in clinical applications.

Metric selection directly addressed clinical priorities. Recall optimization prioritized fracture detection sensitivity to minimize missed diagnoses; a critical imperative given the consequences of delayed treatment. Precision tracking ensured FP rates remained within clinically tolerable thresholds, acknowledging the operational costs of unnecessary follow-up imaging. F1-score balancing provided a composite view of error tradeoffs, while confusion matrices localized vulnerability patterns to specific fracture subtypes. External dataset evaluation explicitly benchmarked cross-institutional generalizability, emulating real-world deployment where models encounter unseen data exhibiting protocol-driven differences.

### 2.7. Implementation details

The entire workflow was implemented using MATLAB R2024b. Preprocessing, model training, cross-validation, and evaluation were performed using MATLAB's Deep Learning Toolbox (version 24.0). The trained model was saved for future use, and confusion matrices were generated for visualizing classification performance.

## 3. Results

The training process of the model is depicted through learning curves, illustrating the evolution of the model's performance over successive epochs (Figure 2). These curves plot both the training and validation loss, providing insights into how well the model is fitting to the training data and how well it generalizes to unseen data. Decreasing loss values indicate improving model performance. In addition, the learning curves display training and validation accuracy, offering a direct measure of the model's classification capabilities on both seen and unseen data. Increasing accuracy values signify improved model performance. By analysing these learning curves, the effectiveness of the training process and the model's capacity to generalize can be assessed.

The performance of the trained model was evaluated on both the validation and test datasets using confusion matrices (Figure 3). These matrices provide a detailed breakdown of the model's predictions by categorizing them into TPs, TNs, FPs, and FNs. This visualization enables a deeper understanding of the types of errors the model makes. For instance, the number of FPs reveals how often the model incorrectly predicts a fracture when there is none, while the number of FNs indicates how often the model misses actual fractures. The accuracy achieved on each dataset, calculated as the ratio of correctly classified samples to the total number of samples, is also presented alongside the confusion matrices, offering a concise summary of the model's performance on these datasets.

*k*-fold cross-validation was employed to evaluate the robustness and generalizability of the trained CNN. The

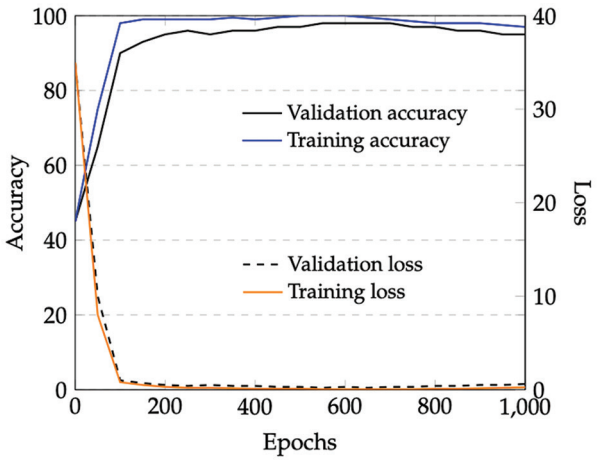


**Figure 2.** Learning curves illustrating the training and validation loss and accuracy over successive epochs. The curves demonstrate the model's convergence during training and its generalization capability on unseen validation data, with decreasing loss and increasing accuracy over time.

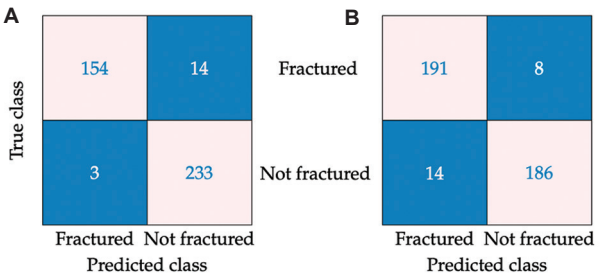


**Figure 3.** Confusion matrices. (A) Confusion matrix for the validation dataset, showing the distribution of true positives, true negatives, false positives, and false negatives. The validation set was used during training to monitor model performance and tune hyperparameters, achieving an accuracy of 95.8%. (B) Confusion matrix for the test dataset, summarizing the model's predictions on unseen data held out during training. The test set accuracy of 94.5% demonstrates the model's strong generalization to new data.

dataset was partitioned into five folds ($k = 5$). The training process was iterated five times, with each fold serving as the validation set while the remaining four folds were used for training. This approach ensured that the model's performance was assessed across different data subsets, mitigating the potential impact of data distribution on the evaluation metrics. The results of each fold's validation were then averaged to obtain a comprehensive measure of the model's performance. Ultimately, the accuracy achieved through $k$-fold cross-validation was 95%.

The generalizability of the model to unseen data was assessed using an external dataset, and its performance is visualized using a confusion matrix (Figure 4). This matrix provides a breakdown of the model's predictions on the external dataset into TPs, TNs, FPs, and FNs, allowing for an evaluation of the model's ability to handle data from a different distribution than the training data. The accuracy of the external dataset was also calculated and presented, providing a quantifiable measure of the model's generalizability. This evaluation is useful for determining the model's real-world applicability.

An overview of the model's performance across different evaluation stages is presented by comparing the accuracy scores obtained on the validation set, through $k$-fold cross-validation, on the test set, and on the external dataset (Figure 5). This comparison assesses the model's consistency and robustness. The validation accuracy reflects the model's performance on a held-out portion of the training data, while the $k$-fold cross-validation accuracy provides a more robust estimate of performance by averaging the results across multiple data splits. The test set accuracy evaluates the model's ability to generalize to unseen data from the same distribution as the training data, and the external dataset accuracy assesses generalizability to data from a different distribution. By comparing these accuracy scores, a comprehensive understanding of the model's performance characteristics can be obtained.

The model demonstrates strong performance across all datasets, with high accuracy, precision, recall, and F1-scores (Table 2). The validation and test datasets show slightly higher performance metrics compared to the external dataset, which is expected due to differences in data distribution. The high recall values across all datasets indicate the model's ability to correctly identify fractures, which is critical in clinical settings to minimize missed diagnoses. However, the slightly lower precision on the external dataset suggests a higher rate of FPs, which could lead to unnecessary follow-up investigations. Overall, the results highlight the model's robustness and potential for real-world application, while also emphasizing the importance of external validation to assess generalizability.

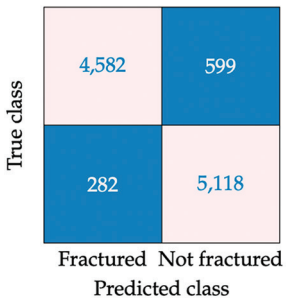


**Figure 4.** Confusion matrix for the external dataset, representing the model's performance on data from a different distribution than the training data. The accuracy of 91.7% demonstrates the model's ability to generalize to diverse clinical scenarios, although with slightly reduced precision.

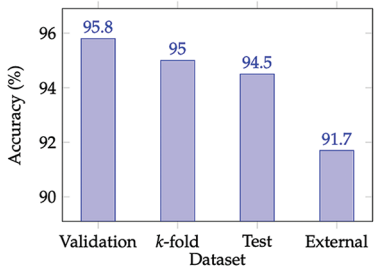


**Figure 5.** Comparison of accuracy values across validation, $k$-fold cross-validation, test, and external datasets. The figure highlights the model's consistent performance on internal datasets and its generalizability to external data, with minimal decline in accuracy.

**Table 2. Performance metrics of the trained convoluted neural network model across datasets**

| Dataset | Accuracy (%) | Precision (%) | Recall (%) | F1-score (%) |
|---|---|---|---|---|
| Validation | 95.8 | 91.7 | 98.1 | 94.8 |
| Test | 94.5 | 95.9 | 93.0 | 94.4 |
| External | 91.7 | 88.4 | 94.2 | 91.2 |

Note: Metrics include accuracy, precision, recall, and F1-score, demonstrating the model's robustness and generalizability with high recall across all datasets and slightly reduced precision on external data.

The trends in Figure 6, which compares the performance metrics (accuracy, precision, recall, and F1-score) across the validation, test, and external datasets, reveal several key insights about the model's performance and generalizability. The validation dataset shows the highest performance across all metrics. This indicates that the model is well-tuned to the training data distribution and performs effectively on data held out during training. The test dataset metrics are slightly lower than those of the validation dataset. This slight drop suggests minimal overfitting and demonstrates the model's ability to generalize to unseen data from the same distribution as the training data. The external dataset shows the lowest

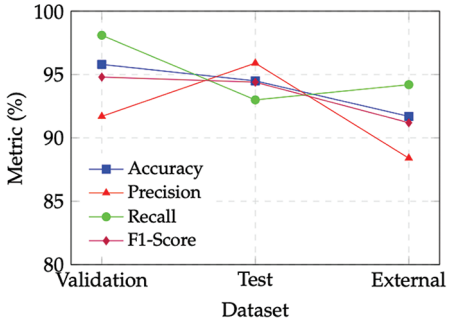


**Figure 6.** Trends in performance metrics (accuracy, precision, recall, and F1-score) for the validation, test, and external datasets. The figure emphasizes the model's robustness and high recall, critical for minimizing missed diagnoses, while precision shows a slight decline on external data due to increased false positives.

performance across all metrics. This drop is expected due to differences in data distribution between the external dataset and the training data. The lower precision indicates a higher rate of FPs, which could lead to unnecessary follow-up investigations in clinical settings.

Across all datasets, recall remains consistently high, with the external dataset achieving a value of 94.2%. This suggests the model is effective at identifying fractures, which is critical in minimizing missed diagnoses. Precision, however, decreases more significantly in the external dataset, highlighting the model's tendency to produce more FPs when applied to data from a different distribution. Hence, the trends as shown in Figure 6 reflect a strong model performance on internal datasets, with a predictable decline in metrics when applied to external data. This emphasizes the importance of external validation and the need for careful consideration of FPs in clinical applications.

## 4. Discussion

The model's diagnostic performance and limitations were analyzed through four critical aspects, assessing how technical achievements in controlled validation translate to clinical utility amidst real-world heterogeneity in imaging protocols, population characteristics, and operational constraints.

### 4.1. Performance and generalizability

The CNN demonstrated robust diagnostic performance, achieving 95.8% validation accuracy and 94.5% test accuracy, with $k$-fold cross-validation ($k$ = 5) confirming stability (95% average accuracy). However, the 8.3% decline in external test accuracy (91.7%) underscores contextual challenges in generalizability. This gradient—from validation to external testing—aligns with the expected impact of domain shift in medical AI, where protocol heterogeneity (e.g., X-ray exposure parameters, sensor resolutions) alters low-level image textures critical for CNN feature extraction.[28] While the model maintained high recall (94.2% externally), precision declined significantly ($\Delta$ = −6.1% relative to test data), reflecting fragility in distinguishing fractures from anatomical mimics (e.g., nutrient canals) under distributional mismatch.

These findings mirror broader ML challenges where aggregate metrics mask subtype-specific vulnerabilities.[9,18] Fracture subtypes underrepresented in training data (e.g., greenstick, pathologic) exhibited higher misclassification rates, emphasizing the need for granular performance reporting across morphological categories. The model's reliance on single-institution datasets—despite $k$-fold validation—limited its capacity to generalize across demographic and regional variations in fracture etiology, echoing concerns in clinical trial generalizability.[5] Future studies should stratify results by fracture type and demographic covariates to better quantify real-world applicability.

While earlier studies in AI-driven fracture detection frequently emphasize high diagnostic accuracy, our results underscore the necessity of evaluating such claims against methodological transparency and validation rigor. Prior research often reports exceptional performance metrics derived from protocols that insufficiently address data leakage or domain heterogeneity—a limitation exemplified by the reliance on single-institution datasets and inconsistent reporting of validation practices. In contrast, our approach prioritized strict separation of training, validation, and testing phases, supplemented by external validation to assess real-world applicability. This framework aligns with recent regulatory guidelines, including the Food and Drug Administration (FDA)'s emphasis on robust validation practices for AI models in clinical settings.[29]

The observed performance gradient between internal and external evaluations reflects methodological divergences (i.e., systematic differences in validation frameworks, data handling, or evaluation criteria across studies) rather than algorithmic shortcomings. While many existing models derive metrics from optimistically partitioned data, our use of cross-validation and architectural safeguards—such as dropout and batch normalization—reduced overfitting risks and maintained training stability. External validation revealed predictable declines in precision, a pattern consistent with domain shift challenges seen across medical AI. These findings highlight a broader issue: nominal accuracy disparities often signal discrepancies in validation practices rather than true model capabilities.

Accordingly, we caution against direct performance comparisons without accounting for differences in evaluation frameworks. Instead, our discussion advocates for harmonized benchmarking standards that prioritize rigorous, clinically representative validation—a prerequisite for bridging the gap between technical achievements and operational reliability in fracture detection.

### 4.2. Cross-study comparison

The above-mentioned inconsistent reporting of validation practices can be seen when analyzing training dynamics in recent studies. For instance, when ML techniques demonstrate inverted learning curves,[30] i.e., the validation accuracy paradoxically exceeds training accuracy, it is a hallmark of methodological flaws, including insufficient data splits, improper hyperparameter tuning, or unaddressed dataset leakage. These inverted patterns, while superficially suggesting high validation performance (e.g., suspicious 99–100% accuracy reports[31]), often mask critical failures in generalizability that only manifest in external testing. Such cases exemplify systemic issues in validation protocols; when models are not stress-tested against distribution shifts or required to demonstrate harmonized training/validation convergence, nominal accuracy metrics become dangerously deceptive proxies for clinical utility. Our methodology directly counters these risks through iterative learning curve monitoring, three-way splitting to eliminate patient data overlap, and architectural safeguards (batch normalization, dropout) explicitly designed to force alignment between training and validation trajectories; a rigor reflected in our model's stable, convergent curves despite more conservative accuracy reporting.

The comparison in Table 3 highlights critical gaps in compliance with FDA-recommended validation protocols, particularly regarding convergence analysis essential for assessing clinical reliability. While FDA's guidance emphasizes harmonized training-validation trajectories as evidence of generalizability,[29] most studies either omit this analysis entirely or present incomplete evidence.[31-33] Among the minority that include learning curves,[30,34] many reveal fundamental inconsistencies; the studies marked ([a]) exhibit inverted validation-training metrics indicating improper data splits or patient overlap, while ([b]) annotations show identical convergence trajectories (i.e., no measurable gap between them). In rigorous ML validation, training metrics should show a slight but consistent divergence from validation metrics; a controlled gap indicating the model is learning without overfitting. When curves are identical, it indicates that the validation set is not truly independent; data from the same patients or images may exist in both training and validation splits. This creates a false impression of perfect generalization, as the model "validates" on data it has effectively memorized during training. This discrepancy demonstrates how insufficient validation reporting, even when nominally including learning curves, permits accuracy inflation through unaddressed data leakage or overfitting while failing to meet regulatory standards for clinical translatability.

Until standardized validation frameworks (e.g., the FDA's guidance emphasizing training-validation convergence) are universally accepted, high reported accuracies in AI-assisted diagnostic studies remain clinically uninterpretable unless accompanied by a demonstration of proper model training and generalizability, as improper convergence risks inflated metrics that invalidate cross-study comparisons.

### 4.3. Error analysis and clinical implications

Error patterns revealed asymmetric risks. FNs predominantly occurred in subtle fractures (e.g., hairline fissures, occult fractures), while FPs arose from anatomical mimics such as trabecular patterns or overlapping soft tissues. This reflects clinical realities where radiologists face similar challenges, though AI may amplify uncertainties due to pixel-space decision-making without anatomical context.

The high recall-low precision tradeoff prioritizes fracture detection sensitivity but risks overutilization of confirmatory imaging (computed tomography/magnetic resonance imaging). For every 100 external cases, approximately 8.3 FPs would necessitate additional investigations, incurring costs and patient anxiety. Conversely, the 5.8% FN rate (versus 3.2% internally) underscores residual risks of delayed treatment, particularly in weight-bearing bones where missed fractures can lead to catastrophic complications. To balance safety and efficiency, clinical deployment should integrate risk-stratified confidence thresholds—lower thresholds for high-stakes anatomical regions (e.g., femoral neck) to maximize sensitivity, and higher thresholds for peripheral sites to reduce unnecessary imaging.

### 4.4. Domain shift and validation best practices

Domain shift emerged primarily from institutional differences in imaging protocols and population characteristics. For instance, external data included a higher proportion of osteoporosis-related fractures, which present distinct morphological signatures (e.g., compressed versus displaced fractures) compared to trauma-driven cases in training data. Protocol variations in beam energy and collimation further degraded performance by altering contrast gradients at fracture edges, a critical CNN

**Table 3. Analysis of validation practices in recent fracture detection studies relative to the Food and Drug Administration's guidance**

| Algorithm | Accuracy (%) | Data splitting | Learning curves | External data testing | Year[Reference] |
|---|---|---|---|---|---|
| MobileNet | 99 | Two-way | Yes, show convergence issues[a] | No | 2025[31] |
| FracNet | 100 | Three-way | No | No | 2025[32] |
| Various | 64–92 | Two-way | No | No | 2023[33] |
| Canny | 90 | Not available | No | No | 2025[34] |
| SimCLR | 94 | Two-way | Yes, show convergence issues[b] | No | 2024[35] |

Note: Analysis of validation practices in recent fracture detection studies relative to Food and Drug Administration's guidance,[30] contrasting reporting gaps in training dynamics and external generalizability. Methodologies frequently omit evidence of training-validation convergence and external performance benchmarking. Many report inflated accuracy metrics using internal validation via two-way splitting, which obscures patient overlap risks and prevents cross-study comparability of clinical utility. Notably absent are learning curves demonstrating harmonized training dynamics or stress-testing against distribution shifts, undermining confidence in real-world reliability. While some studies include learning curves, these often reveal erratic validation trajectories indicative of improper regularization or dataset leakage. [a]refers to inverted learning curves, and [b]refers to identical convergence trajectories.
Abbreviation: SimCLR: A simple framework for contrastive learning of visual representations.

input.[35] To mitigate these effects, we advocate multicenter validation frameworks that: (i) Prospectively harmonize imaging protocols across sites using Digital Imaging and Communications in Medicine metadata standardization, (ii) Implement continuous test-time adaptation via adversarial domain-invariant training,[36] and (iii) Adopt federated learning architectures to pool heterogeneous data while preserving institutional privacy.[37]

Our results demonstrate that conventional single-center holdout validation—even with rigorous *k*-fold splits—overestimates real-world performance by up to 11.3% (external vs. best-case validation accuracy). This aligns with the emerging consensus that external validation should precede clinical implementation, supplemented by stress-testing against rare but critical edge cases (e.g., pediatric buckle fractures).

### 4.5. Limitations and future directions

While the model demonstrated robustness across two independent datasets, three inherent limitations merit clarification. First, this study did not curate or harmonize patient ages, as neither source dataset included demographic metadata. This reflects real-world clinical deployments where AI tools process images without comprehensive patient histories, prioritizing fracture morphology over population characteristics. Second, the underrepresentation of pathological fractures (those arising from underlying disease processes like metastatic cancer or osteoporosis) versus traumatic fractures (mechanical injuries in structurally normal bone) poses a distinct challenge. Third, despite multi-dataset integration, sample scarcity persists for pediatric (<18 years) and older (>65 years) populations, an unavoidable constraint given their smaller population proportions (~30% collectively). Naturally, the fact that under-18 and over-65 age groups collectively make up about 30% of the population (based on United States census data[38]) inevitably leads to a dataset composition that reflects this distribution.[39] Future work should explicitly recruit these cohorts to assess performance across developmental and degenerative bone phenotypes. Hence, key limitations include:

(i) Dataset diversity gaps: Underrepresentation of pediatric/geriatric populations and pathologic fractures. However, clinical applicability depends on generalizability across institutions, not demographic alignment. Including age-specific tuning could paradoxically reduce robustness by overfitting to non-generalizable population features
(ii) Label noise: Retrospective ground truth from clinical reports inherits inter-observer variability, with up to a 14% discordance rate (the proportion of cases where annotators disagreed on fracture presence) in subtle fracture annotation[1]
(iii) Operational fragility: Performance degrades when faced with non-standard views (e.g., oblique projections) not included in training.

To address these, future work should (i) Develop synthetic data augmentation pipelines tuned to rare fracture phenotypes using diffusion models,[40] (ii) Implement triple-annotation protocols with orthopedist adjudication to minimize label noise, and (iii) Integrate attention mechanisms focusing on cortical discontinuity (interruption of bone cortex) and periosteal reactions (bone healing responses)—morphological hallmarks less sensitive to imaging artifacts.

In addition, the "black box" nature of CNNs and deep NNs limits interpretability, which may hinder clinical trust and impede its seamless integration into diagnostic workflows.[36] Finally, the retrospective design of this study

introduced potential selection biases and constrained the assessment of model performance in prospective, real-world applications, suggesting that further research is necessary to refine the system for use as a screening tool rather than a definitive diagnostic instrument.

Collaborative learning across trauma networks could enhance generalizability while adhering to data governance constraints, though this requires standardized annotation frameworks to ensure cross-site label consistency. Ultimately, progression to clinical utility demands co-design with radiologists to align AI outputs with interpretable diagnostic criteria.

## 5. Conclusion

While the model demonstrated high overall accuracy, the clinical implications of FPs and FNs warrant careful consideration. FPs, where the model incorrectly identifies a fracture, can lead to unnecessary further investigations, increased patient anxiety, and potential delays in appropriate treatment for the actual underlying condition. For example, an FP might trigger additional imaging studies, such as computed tomography scans or magnetic resonance imaging, which expose patients to radiation or contrast agents and add to healthcare costs. Conversely, FNs, where the model fails to identify a true fracture, pose a more serious risk. Missed fractures can result in delayed or inadequate treatment, potentially leading to long-term complications such as malunion, nonunion, or chronic pain. Moreover, in weight-bearing bones, a missed fracture could lead to further injury and disability. Therefore, while AI can be a valuable tool, clinicians should always interpret the model's output in conjunction with their own clinical judgment, patient history, and other diagnostic information to minimize the impact of both FPs and FNs.

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The authors declare they have no competing interests.

## Author contributions

*Conceptualization:* All authors
*Formal analysis:* All authors
*Investigation:* All authors
*Methodology:* All authors
*Writing–original draft:* All authors
*Writing–review & editing:* All authors

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data

All data analyzed have been presented in the paper.

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ORIGINAL RESEARCH ARTICLE

# Technique sensitivity hampers outcomes in periodontal regeneration when performed by less experienced operators: A retrospective analysis

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## Abstract

Flap design is a key factor in the clinical outcomes of periodontal regeneration (PR). This study compares the effectiveness of minimally invasive flap (MIF) to conventional flap (CF) techniques in PR procedures performed by periodontic residents. The study also addresses how technique sensitivity may influence clinical outcomes when performed by less experienced operators. A retrospective study was conducted on patients who underwent PR from January 2012 to January 2023 at the School of Dentistry, University of Michigan. Flap techniques were classified as MIF or CF, and clinical outcomes, including bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL), gingival recession (GR), changes in keratinized gingiva, and tooth loss, were evaluated. Statistical analysis using generalized estimation equations was performed for the overall sample and separately for each group. The study sample consisted of 40 male (45.5%) and 48 female patients (54.5%), with an average age of 63.1 ± 13.8 years and a mean follow-up of 42 months. No significant differences were found between the MIF and CF groups regarding the reduction in PD or GR. However, the CF group exhibited a superior gain in CAL ($p$=0.005) and a greater decrease in BOP after adjustment for confounders (odds ratio: 4.44, $p$=0.0276). Tooth type and defect depth were identified as significant factors affecting clinical outcomes. Both techniques were effective in treating periodontal defects. However, the CF approach demonstrated a greater improvement in CAL and BOP. Given the technique-sensitive nature of MIF, the limited clinical experience of resident operators may have contributed to the diminished performance of MIF observed in this study. Simpler surgical techniques may offer comparable effectiveness to more complex, superior surgical techniques in a university-based setting when performed by less experienced operators.

***Keywords:*** Guided tissue regeneration; Periodontal disease; University-based services; Smoking

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## 1. Introduction

Periodontal regeneration (PR) is a valuable treatment modality for managing isolated furcation and intrabony defects (IBD).[1,2] Several systemic and localized factors are considered well-evidenced risk factors and predictors of periodontal and peri-implant therapy success.[3-5] In addition, numerous clinical studies have explored the impact of various membranes, bone grafts, and biological agents on PR success.[6-8] Despite these variables, the clinician's skill in several aspects of the procedure—such as case selection, incision and flap design optimization, supra-crestal tissue compartment management, materials selection, membrane manipulation, and type of suturing techniques—directly influences the healing process. Ultimately, these skills impact the outcomes of the PR procedure.[9] Therefore, ongoing refinement of skills and adopting technological innovations are essential for achieving the best possible results for patients.[10]

The conventional flap (CF) technique, involving buccal and lingual/oral flap reflection beyond the limits of IBD,[11] is commonly used due to its convenience and ease of execution.[12,13] However, recognizing the importance of effective access to the interdental blood supply, wound closure, and optimal tissue/bone regeneration, various surgical approaches have been developed.[14] Minimally invasive flap (MIF) techniques aim to limit tissue reflection, thereby reducing surgical trauma and lowering the risk of post-operative complications, the most common of which is membrane exposure.[15-20] Notable examples include the papilla-preserving flap technique introduced by Takei in 1985,[21] its modifications by Cortellini in 1995 and 1999,[15,16] the minimally invasive surgical technique (MIST),[22-24] and its later modification, the modified MIST (M-MIST).[18] In treating deep IBD, using an MIF is crucial to optimize wound stability, maintain flap integrity, and promote primary intention healing in molars.[25,26]

Studies have investigated the outcome of surgical and non-surgical procedures performed by inexperienced clinicians, such as university-based periodontal residents.[27-29] Brayer *et al.*[27] reported that root debridement performed by 2nd-year periodontal residents compared to fully trained American board-certified, experienced level 1 clinicians was less effective. In a retrospective analysis, Kozlovsky *et al.*[28] concluded that the operator's experience level will affect the outcome and the patient's compliance. Similarly, Ozcan *et al.*[29] compared the reported clinical and esthetic results of coronally advanced flap procedures for the treatment of gingival recession (GR). The 6-month results showed that an advanced surgical experience level results in higher percentages of root coverage. In addition, as the experience level increased, the rate of complications and the operative time decreased.

There are currently no reports in the literature assessing the efficacy of technique-sensitive procedures, such as MIF, compared to more straightforward procedures, such as CFs for PR in inexperienced clinicians. Therefore, the study aims to evaluate the outcomes of using MIF incisions compared to CF in PR procedures performed by less experienced periodontal residents while considering systemic risk factors/predictors of included subjects, hypothesizing that MIF should result in more predictable outcomes compared to CF, consistent with the available literature.[15,16]

## 2. Methodology

### 2.1. Study population

Patient data of those who underwent periodontal therapy from January 2012 to January 2023 at the School of Dentistry, University of Michigan, in Ann Arbor, Michigan, United States, were retrieved from electronic health records. The research received ethical clearance from the Institutional Review Board of the University of Michigan (IRBMED: HUM00248789).

### 2.2. Data collection

#### *2.2.1. Inclusion criteria*

The inclusion criteria are as follows:

(i) Individuals aged ≥18 years
(ii) Individuals diagnosed with IBD ≥ 5 mm probing depth (PD) of vital anterior, pre-molar, or molar, with or without furcation involvement, treated with PR in the post-graduate periodontics department at the University of Michigan.
(iii) Individuals with baseline and follow-up periodontal charts with complete clinical parameters (clinical attachment level [CAL], PD, bleeding on probing [BOP]) and comprehensive clinical notes describing the PR procedure in detail, indicating the type of flap used and the materials utilized.
(iv) Patients compliant with supportive periodontal therapy following surgeries.

#### *2.2.2. Exclusion criteria*

The exclusion criteria are as follows:

(i) Individuals with hopeless teeth (Based on the definition provided by Sanz *et al.*[30]).
(ii) Individuals with systemic conditions that were generally considered to be contraindications to periodontal surgery, but not limited to severe osteoporosis, uncontrolled diabetes, and blood dyscrasias.

(iii) Individuals who were pregnant or lactating.
(iv) Individuals who did not have baseline and/or follow-up complete periodontal charts or clinical notes, especially those not specifying the flap technique used for PR.

#### *2.2.3. Flap technique description*

Two flap techniques were investigated in this study: (i) MIF and (ii) CF techniques. MIF technique is a general term describing conservative flap reflection to the bony limits of the defect or to single-flap designs. This study included the modified papilla preservative incision technique,[16] the simplified papilla preservation (SPPF) incision technique,[15] and the entire papilla preservation technique.[18,25,31] Figure 1 illustrates the SPPF technique. Results of subgroup analysis are presented in Table S1. CF technique consists of a buccal and lingual/oral flap reflection beyond the limits of the IBD, usually a horizontal crestal incision with or without vertical releasing incisions to reflect a full-thickness or partial-thickness flap.[11,13] Figure 2 illustrates the CF technique.

Two independent evaluators collected the data retrospectively of two patient cohorts from previously treated PR cases: The experimental group, in which incisions were performed using the MIF designs, and the control group, in which the CF approach was employed. For both groups, a comprehensive review of available clinical records was conducted regarding the specific incision and flap techniques employed, the type of membrane and graft used, the biological agents utilized, and all post-operative complications encountered.

At the patient level, demographics captured included patient sex and age, while general health data encompassed the presence of diabetes mellitus at the onset of PR therapy and smoking history categorized as non-smoker, former smoker, or current smoker (along with daily cigarette consumption). Each patient's periodontal status was classified using the staging and grading system for periodontitis, which includes stage (1–4), grade (A, B, and C), and extent (localized, generalized), as defined immediately before PR treatment.[32] In addition, the analysis considered the duration of each patient's follow-up as well as the frequency of maintenance visits after the procedure.

At the tooth level, tooth-specific clinical parameters, such as PD, CAL (formerly calculated and recorded in the chart as the difference between PD and the distance from the free gingival margin to the cementoenamel junction), BOP, and tooth mobility, were assessed alongside the width of keratinized tissue. Furcation involvement was examined for the molar tooth.[33] Two time points were considered

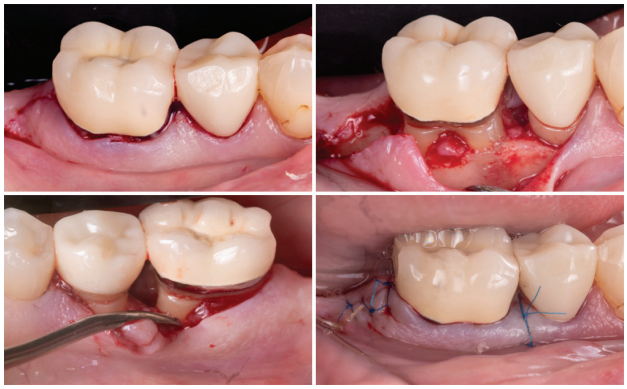

**Figure 1.** Representation of the simplified papilla preservation flap performed by a periodontics resident

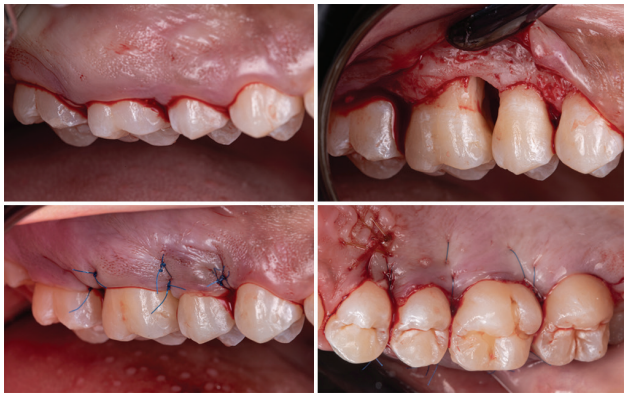

**Figure 2.** Representation of the conventional flap technique performed by a periodontics resident

in data collection: T0, indicating the baseline period before surgical intervention, and T1, representing the past follow-up chart, at least 12 months after the procedure. Intra-operative details specifically focused on the dimensions and morphology of the periodontal defects, the bone graft used, and the type of membrane used in the PR procedure, if any. Any biological agents deployed, antibiotics prescribed, and post-operative complications, including infections or membrane exposures, were collected.

For operators, their levels were determined by the year of the procedure and the corresponding resident level. R1 was designated as 1st-year residents, R2 as 2nd-year residents, and R3 as 3rd-year residents.

### 2.3. Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences software (version 22.0, IBM, United States). The following clinical parameters were considered primary outcomes: BOP, PD reduction, CAL gain, keratinized gingiva (KT) change, GR at T1, and

tooth loss due to periodontal reasons (TLP).[34] At the tooth level, multilevel binary logistic regression with generalized estimation equations was used to relate the independent factors and covariates to the binary outcomes (e.g., TLP, BOP at T1). Raw odds ratio (OR) and 95% confidence intervals (CI) were obtained from the Wald's $\chi^2$ statistic. Then, multiple models were estimated to adjust by potential confounding factors (at patient's level, such as age, sex, smoking, diabetes mellitus, periodontitis diagnosis, number of maintenance visits, and duration of follow-up, as well as clinical data, including the number of walls, antibiotic use, the type of membrane applied, furcation involvement, number of walls of the defect, defect dimensions, and the type of grafting material). Quantitative outcomes (e.g., CAL gain, PD reduction, KT change, and GR at T1) were analyzed using linear regression models estimated with generalized estimation equations to control the within-subject dependence of teeth. Beta coefficients and 95% CIs were reported. As previously mentioned, multiple models were estimated. The significance level used in the analysis was 5% ($\alpha = 0.05$).

To ensure sufficient statistical power to detect a clinically valuable difference between the MIF and CF groups, a power analysis using a *post hoc* estimation was performed. A sample size of 99 independent teeth provides 72.3% power at a 95% confidence level to detect an OR of 3 as significant using a logistic regression model (an OR of 3 is equivalent to comparing rates of 50% and 25%, for example, for BOP rates). However, since teeth were not independent observations, the power was adjusted to account for the two-level structure of the data. Each patient provided an average of 1.13 teeth. Assuming a moderate within-subject correlation of 0.5, a correcting coefficient of 1.06 was obtained. Therefore, a sample of 99 dependent teeth was equivalent to 93 independent observations, yielding an estimated power of 70.0% under the same previous conditions.

## 3. Results

### 3.1. Characteristics of the study population

There were 40 males (45.5%) and 48 females (54.5%), with an average age of 63.1 ± 13.8 years, ranging from 30 to 87 years at baseline. Each patient contributed, on average, 1.13 teeth to the database, resulting in a total sample of 99 teeth (40 in the MIF group and 59 in the CF group) treated by 84 post-graduate residents. Table 1 summarizes the demographic characteristics of both groups, the type and location of tooth, and the operator level.

The sample included 88 patients who underwent either MIF ($n$ = 36, consisting of 30% SPPF incision technique, 47.5% modified papillary preservation incision technique, and 22.5% papillary preservation incision technique) or CF ($n$ = 52) procedures. The mean follow-up period after treatment was 42.0 ± 30.1 months, ranging from 2 to 163 months (median: 34; interquartile range: 19–57).

In terms of group homogeneity, no significant differences were found between the groups regarding patient-level covariates, such as sex, age, diabetes mellitus, smoking, and periodontitis diagnosis (staging and grading), as well as the number of follow-up visits ($p$>0.05). Group homogeneity information is presented in Table S2.

No significant difference was found in the treating residents' level, with 2nd-year residents predominately contributing to both groups (MIF: 47.6%, CF: 52.3%).

### 3.2. Analysis of changes in clinical outcomes

#### *3.2.1. Effect of procedure complexity on clinical outcomes*

The mean CAL gain was found to be 2.17 ± 2.18 mm for the CF group, in contrast to only 0.59 ± 3.43 mm for the MIF group (Figure 3). Significant differences in CAL gain remained between the groups even after adjusting

**Table 1. Demographic characteristics of both groups, the type and location of the tooth, and the operator level**

| Demographic characteristics | Total | CF | MIF |
|---|---|---|---|
| Number of patients | 88 | 52 | 36 |
| Age, mean (years) | 63.1±13.8 | 63.16 | 63.45 |
| Sex (M/F) | 40/48 | 25/28 | 15/20 |
| Follow-up time, mean (months) | 42.0±30.1 | 44.85 | 38.5 |
| Number of teeth | 99 | 59 | 40 |
| Type of the tooth (%) (M/P/C/I) | 65.7/19.2/4.0/11.1 | 86.4/6.8/5.1/1.7 | 35.0/37.5/2.50/25.0 |
| Arch (%) (Max/Man) | 44.3/55.6 | 62.5/37.5 | 32.2/67.8 |
| Operator level (R1, R2, R3) | 13, 42, 29 | 9, 22, 19 | 4, 20, 10 |

Note: R1, R2, and R3 refer to 1st-year residents, 2nd-year residents, and 3rd-year residents, respectively.
Abbreviations: CF: Conventional flap; M/F: Male/female; M/P/C/I: Molar/premolar/canine/incisor; Max/Man: Maxilla/mandible; MIF: Minimally invasive flap.

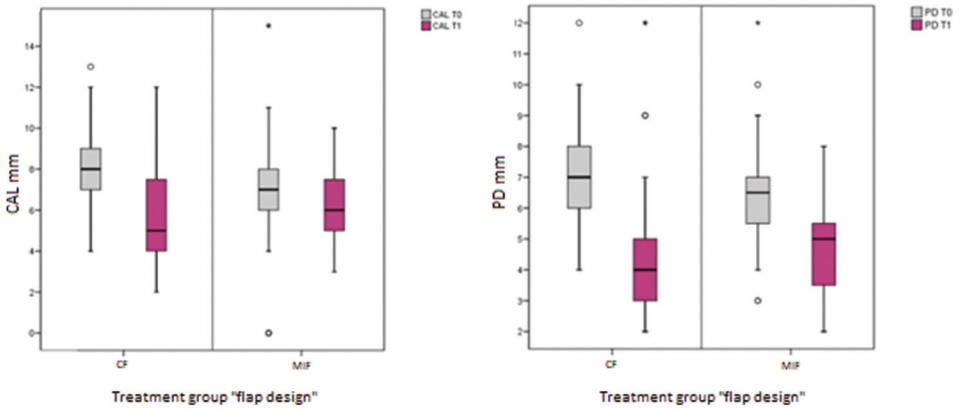


**Figure 3.** CAL gain and PD reduction for MIF and CF at T0 and T1. (A) CAL gain; (B) PD reduction. The mean CAL gain decreased by 2.17 ± 2.18 mm from T0 to T1 for the CF group, in contrast to only 0.59 ± 3.43 mm for the MIF group. T0 and T1 refer to the 1st and 2nd time points, respectively
Abbreviations: CAL: Clinical attachment level; CF: Conventional flap; MIF: Minimally invasive flap; PD: Probing depth.

for confounding factors ($p$<0.001), indicating a greater CAL gain for the MIF group (Tables 2 and S3). Both the MIF and the CF groups showed improvements in PD and BOP at the follow-up. However, no significant differences were observed between the two groups in PD reduction ($p$=0.218) (Table 3). BOP was slightly higher in the MIF group at T1 ($p$=0.047) (Figure 4). Similarly, for GR, no statistically significant difference was found between the CF and MIF groups ($p$=0.073) (Tables S4-S7).

#### *3.2.2. Effect of covariates on clinical outcomes*

The depth of the defect was a critical factor, as it negatively impacted the CAL gain outcome ($p$=0.003) (Table 2). Teeth in the Stage 3 or 4 patients resulted in higher PD reductions compared to Stage 1 patients ($p$=0.003, Table S8). Deeper and larger defects were correlated to less PD reductions ($p$=0.001 and $p$=0.020, respectively) (Tables 3 and S8).

### 3.3. Analysis of tooth loss due to periodontitis

MIF and CF showed the same TLP rate ($p$=0.521). A more significant occurrence of TLP was noted in teeth with furcation involvement, regardless of the flap design (OR: 4.3, $p$=0.035).

## 4. Discussion

The study investigated the effectiveness of two flap designs, MIF and CF, in treating isolated intra-bony and furcation defects within a periodontics residency program. Both techniques were effective in treating periodontal defects. No statistically significant differences were found between the two groups regarding PD, GR, or TLP. Nonetheless, notable differences emerged between the MIF and CF groups in terms of CAL gain and BOP reduction.

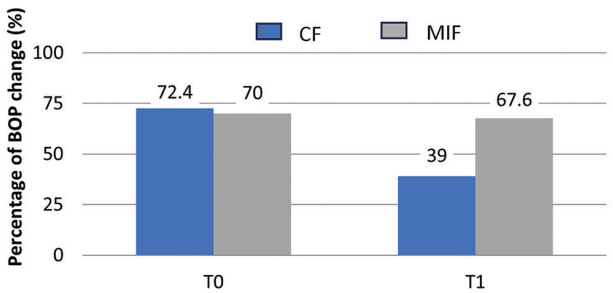


**Figure 4.** Percentage of BOP changes by group. The CF group demonstrated improved BOP changes compared to the MIF group from T0 to T1. A total of 41.4% of teeth reported an improved BOP status in the CF group, but only 17.6% in the MIF group. T0 and T1 refer to the 1st and 2nd time points, respectively.
Abbreviations: BOP: Bleeding on probing; CF: Conventional flap; MIF: Minimally invasive flap.

Considering the CAL gain, several factors contributed to the superior gain in the CF group compared to the MIF group. The insufficient gingival phenotype (keratinized width and thickness) that was found to be significantly lower in the MIF group can negatively impact the results. According to Tonetti *et al.*[3,35] and a recent review by Levine *et al.*,[3,35] a thin gingival phenotype appears to be at greater risk of exhibiting GR in response to regenerative procedures than a thick phenotype. The keratinized tissue width appears to play a role in flap stability and flap micromotion prevention, affecting healing. De Ry *et al.*[36] presented 10-year follow-up results of PR with enamel matrix derivatives, reporting that maxillary molars were correlated with an increased risk for CAL loss. This aligns with the findings in our cohort, with the CF group mostly in the mandibular arch compared to the MIF group.

**Table 2. Multiple linear regression using generalized estimation equation model estimation of clinical attachment level gain (T0–T1) by independent factors and covariates**

| Factors | β | 95% confidence interval | *p*-value |
|---|---|---|---|
| Group | | | |
| CF | 0 | - | - |
| MIF | −1.65 | −2.48–−0.82 | <0.001*** |
| Stage | | | 0.069 |
| 2 | 0 | - | - |
| 3 | −1.47 | −2.81–−0.14 | 0.031* |
| 4 | −1.90 | −3.57–−0.22 | 0.027* |
| Position | | | 0.010* |
| Anterior | 0 | | |
| Premolar | 1.07 | −0CF.09–2.04 | 0.032* |
| Molar | −0.47 | −1.35–0.41 | 0.291 |
| Arch | | | |
| Maxilla | 0 | - | - |
| Mandible | −0.45 | −1.13–0.22 | 0.188 |
| No. of walls | | | 0.305 |
| 1 | 0 | - | - |
| 2 | −0.74 | −1.86–0.39 | 0.198 |
| 3 | 0.23 | −0.92–1.37 | 0.701 |
| Defect depth | −0.24 | −0.39–−0.08 | 0.003** |
| Antibiotics | | | |
| No | 0 | - | - |
| Yes | −0.31 | −1.11–0.49 | 0.451 |
| CAL T0 | 0.67 | 0.53–0.81 | <0.001*** |
| KT T0 | | | |
| No | 0 | - | - |
| Yes | −0.14 | −0.96–0.67 | 0.733 |

Notes: Statistical significance determined at *$p$<0.05, ** $p$<0.01, and ***$p$<0.001. T0 refers to the 1st time point.
Abbreviations: CAL: Clinical attachment level; CF: Conventional flap; KT: Keratinized gingiva; MIF: Minimally invasive flap.

**Table 3. PD reduction (T0–T1) by independent factors and covariates**

| Factors | β | 95% confidence interval | *p*-value |
|---|---|---|---|
| Group | | | |
| CF | 0 | - | - |
| MIF | −0.41 | −1.07–0.24 | 0.218 |
| Stage | | | 0.730[a] |
| 2 | 0 | - | - |
| 3 | 0.34 | −0.62–1.31 | 0.484 |
| 4 | 0.58 | −1.04–2.20 | 0.483 |
| Position | | | 0.014* |
| Anterior | 0 | - | - |
| Premolar | 0.05 | −0.74 – 0.85 | 0.896 |
| Molar | −0.77 | −1.55–0.02 | 0.056 |
| Arch | | | |
| Maxilla | 0 | - | - |
| Mandible | 0.28 | −0.29–0.85 | 0.331 |
| No. of walls | | | 0.843 |
| 1 | 0 | - | - |
| 2 | −0.23 | −1.08–0.63 | 0.608 |
| 3 | 0.03 | −0.79–0.85 | 0.331 |
| Defect depth | −0.25 | −0.39–−0.11 | <0.001*** |
| Defect width | 0.00 | −0.14–0.14 | 0.997 |
| Antibiotics | | | |
| No | 0 | - | - |
| Yes | −0.31 | −0.92–0.31 | 0.331 |
| No. of furcation | | | |
| 0 | 0 | - | - |
| ≥1 | −0.44 | −1.17–0.29 | 0.241 |
| PD T0 | 0.65 | 0.47–0.83 | <0.001*** |
| KT T0 | | | |
| No | 0 | - | - |
| Yes | −0.39 | −1.05–0.27 | 0.245 |

Notes: Statistical significance determined at *$p$<0.05, **$p$<0.01, and ***$p$<0.001. T0 refers to the 1st time point.
Abbreviations: CAL: Clinical attachment level; CF: Conventional flap; KT: Keratinized gingiva; MIF: Minimally invasive flap; PD: Probing depth.

The technique sensitivity of the MIF procedures, which requires a more experienced operator and proper magnification, might contribute to less CAL gain in this group, assuming it was sub-optimally executed by training residents, therefore leading to less favorable outcomes. The initial defect depth was found to affect CAL gain outcome significantly, aligning with the findings of Tonetti *et al.*[37] However, no significant difference was found in the initial defect depth between the groups.

Regarding the PD reduction, there was no significant difference between the CF and MIF groups, with molar teeth showing less improvement compared to pre-molar and anterior teeth. Nibali *et al.*[26] stated that papilla preservation flaps yield superior outcomes and should be regarded as an essential surgical step in any regenerative procedure that aims to achieve better PD results. Our finding of no difference in PD reduction is aligned with a study by Windisch *et al.*,[38] which also reported no differences in resulting PD reduction irrespective of the employed surgical technique for IBDs treatment with enamel matrix derivatives.

Both groups demonstrated improvements in BOP, with significantly greater reduction in BOP in the CF group

than the MIF group at T1, as reported by the multiple regression model. This indicates a higher level of persistent inflammation in the MIF group in our cohort, which is mainly located in the maxil.[39] This aligns with a previous study by De Ry *et al.*,[36] which reported that maxillary molars are more prone to complications than mandibular molars.

GR improvement was observed in both groups, with the CF group showing slightly better, but insignificant, improvement compared to the MIF group at T1. This might be attributed to the difference in gingival phenotype discussed earlier. Some relapse might also be expected over the follow-up period. In our cohort, longer follow-up periods were associated with a higher incidence of GR.

The robustness of our investigation lies in its comprehensive examination of the effectiveness of surgical methodologies in facilitating PR, juxtaposed with the operator's proficiency level. All patients in this study were compliant with supportive periodontal therapy. In addition, our analysis encompassed consideration of various systemic and localized variables pertinent to the procedure. However, the retrospective nature of the study limits our ability to establish a causal relationship between flap design and treatment outcomes. Prospective, randomized controlled trials would provide more substantial evidence, especially when considering novel techniques for gingival augmentation, as well as cell technologies, collagen matrices, mucoderm, mucograft, gene therapy, and gene-activated materials.[40,41] Furthermore, the majority of the teeth involved were molars. Nevertheless, there were no differences attributable to technique between different tooth locations or between furcation and non-furcation teeth. In addition, the study's reliance on a university-based environment may introduce selection bias, potentially affecting the generalizability of the findings. Despite attempts to adjust for confounding variables, other unmeasured factors may influence treatment outcomes, oral hygiene habits, and systemic health conditions. The variability in follow-up duration, ranging from 12 to 163 months, may introduce bias and affect the interpretation of long-term treatment outcomes. The study observed a varied distribution of flap techniques intraorally, which may introduce heterogeneity in treatment outcomes. Standardization of treatment protocols could enhance the reliability of comparisons between groups. While the study evaluated several clinical parameters, other important factors, such as patient-reported outcomes and radiographic assessments, were not included due to the lack of systematic collection of such data. This limited the comprehensiveness of the analysis.

Our study was conducted in a university-level setting, where the procedures were executed by residents under the supervision of a faculty member, potentially affecting the results. Lizio *et al.*[42] concluded that the level of the operator's expertise is relevant in conditioning the final results. Yet, the study provides valuable insights into PR efficacy, particularly for those in training or with limited experience. Both MIF and CF techniques were effective in treating periodontal defects. These findings can guide periodontists in selecting the most appropriate surgical techniques based on individual patient needs and clinical goals, ultimately enhancing overall outcomes.

Studies with more standardized protocols of higher sample sizes might be needed to further evaluate the efficacy of MIF techniques compared to CF on PR outcomes, especially in treating posterior multirooted teeth.

## 5. Conclusion

Both MIF and CF techniques conducted by periodontic graduate residents within a university-based setting effectively treated periodontal defects. However, the CF approach demonstrated superior improvements in CAL and BOP. In addition, simpler flap designs can offer comparable outcomes to more complex techniques conducted by less experienced operators in less-than-ideal clinical situations.

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## Conflict of interest

The authors declare that they have no competing interests.

## Author contributions

*Conceptualization:* Hamzeh Almashni, Abdusalam Alrmali
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*Project administration:* Hamzeh Almashni
*Writing – original draft:* Hamzeh Almashni, Ruben Leyton, Abdusalam Alrmali
*Writing – review & editing:* Muhammad H. A. Saleh, Hom-Lay Wang

## Ethics approval and consent to participate

The Institutional Review Board (IRB) for the Medical Sciences at the University of Michigan, Ann Arbor, MI, reviewed and approved this study before enrollment of participants began (IRBMED: HUM00248789). Informed consent was obtained from all participants before participation in the study.

## Consent for publication

Participants consented to the publication of their data.

## Availability of data

The original raw data collected are available from the corresponding author upon reasonable request.

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ACCSCIENCE PUBLISHING

Global Translational Medicine

ORIGINAL RESEARCH ARTICLE

# CoreView imaging on needle: Rapid core-needle biopsy imaging for point-of-care breast cancer diagnosis

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## Abstract

Breast cancer (BC) is one of the most prevalent malignancies worldwide, with early and rapid diagnosis playing a critical role in improving patient outcomes. Core-needle biopsies (CNBs) are the current gold standard for minimally invasive BC diagnoses. However, in low-resource and rural settings, access to CNB diagnostics is limited by infrastructural constraints, long histopathology turnaround times, as well as financial and geographical barriers. To address these challenges, we developed the CoreView imaging on needle (ION), an affordable, integrated imaging system designed to provide rapid, and point-of-care diagnostic assessment of CNB samples. The CoreView ION integrates microscopy with ultraviolet surface excitation technology, enabling the imaging of tissue biopsy surfaces within 5 min, significantly reducing diagnostic delays. This study presents the design, fabrication, and verification of the CoreView ION prototype operation, including its imaging workflow, staining protocols, and tissue compression testing. Our results demonstrate that the system can successfully generate histology-grade images of porcine and murine fresh biopsies, preserving cellular and nuclear detail of normal and tumor tissue. By streamlining CNB imaging and incorporating mainly manual low-cost components, the CoreView ION has the potential to improve BC diagnostics in low-resource settings, ultimately enhancing early detection and patient care.

***Keywords:*** Microscopy with ultraviolet surface excitation imaging; Point-of-care; Global health; Breast cancer; Diagnostics

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## 1. Introduction

Breast cancer (BC) is characterized by dysregulated cell proliferation within breast epithelium, with common types being invasive ductal or invasive lobular carcinoma.

Globally, BC affects 2.8 million individuals annually, causing approximately 690,000 deaths.[1,2] Early diagnosis is crucial for improved patient outcomes, as it significantly impacts an individual's quality of life and ability to combat the disease. Despite recent advances in preventive treatment measures, BC remains highly prevalent, with one in eight women likely to develop the disease in their lifetime in the United States alone, making it a major focus for treatment improvement.[3] In low-resource rural settings and low- and middle-income countries (LMICs), BC care presents various challenges and has a much worse 5-year survival rate compared to higher-income countries (HIC).[4,5] In the United States, for example, the 5-year survival rate is 83.9%; however, in LMICs, such as Gambia, it is as low as 12%.[6] Overall, 58% of BC deaths occur in LMICs, underlining the critical need for improved diagnostic and treatment methods.[6] In Ghana specifically, BC is the most common cause of cancer death for Ghanaian women.[7] The main reason is the late presentation of patients and diagnostic delays.[3-7] With the introduction of new diagnostic devices, differences in patient care globally can be minimized.

### 1.1. Current clinical practice in BC diagnosis

In current clinical practice, two primary methods are commonly used for minimally invasive tissue sampling: fine-needle aspiration (FNA) and core-needle biopsy (CNB).[8] While both techniques involve the extraction of cellular material using a needle, they differ in procedure, diagnostic efficacy, and clinical utility. FNA samples utilize smaller needle gauges (22–25 gauge); however, they constitute isolated cells and cell clumps without tissue architecture and thus can be suboptimal for diagnostics, requiring the expertise of a trained cytopathologist to ensure accurate analysis. In contrast, CNB utilizes a larger gauge needle (14–20 gauge) equipped with a spring-loaded cutting mechanism to excise tissue samples from suspected tumors, providing superior diagnostic accuracy, specificity, and sensitivity.[9]

The larger and more structurally intact tissue samples obtained through CNB facilitate histopathological evaluation, making it the standard of care for BC diagnostics.[9] Despite their diagnostic advantages, CNBs are associated with higher procedural costs and require time-intensive histopathological tissue processing workflows that contribute to delays in BC diagnosis and treatment (Figure 1). In the United States, current trends involve pathologists seeking to optimize the biopsy process by reducing the number of samples required for an accurate diagnosis. Historically, patients underwent 5–10 CNBs per procedure; however, recent studies have indicated that 3–5 cores are adequate for diagnostic or clinical management, and even as few as 2 cores may reliably allow for diagnosis of a malignancy.[10]

### 1.2. Challenges in BC diagnosis in low-resource settings

BC remains a major public health challenge in LMICs, where resource limitations significantly impact diagnosis and treatment. A major constraint is the shortage of essential equipment, inadequate organizational infrastructure, and an insufficient number of qualified personnel within pathology and lab medicine (PALM) services in such areas. PALM services are crucial for accurate disease detection and prognosis; without such services, patients are often uninformed for extended periods without a definitive diagnosis.[11] Similar challenges are also observed in remote and rural areas of HICs from a lack of funding and continual closing of rural hospitals, restricting access to PALM services.[11,12]

Although CNB plays a critical role in BC diagnosis and treatment planning, the clinical procedure is often constrained by barriers, such as a shortage of trained professionals to precisely acquire cores from the targeted mass, as well as the equipment and supplies required to process the specimens in adequately equipped histology facilities.[13] More sophisticated techniques, including ultrasound imaging and vacuum-assisted breast biopsy techniques, are not widely implemented due to cost considerations.[14] Logistical constraints also hinder histopathological processing in LMICs. In low-resource settings, formalin-fixed paraffin-embedded (FFPE) tissue processing and pathologist diagnosis can take up to 3 months to complete, compared to approximately 1 week in high-resource settings.[15] In Ghana's eastern region, the lack of local pathologists exacerbates delays, as samples must be sent off-site for evaluation.[16]

Early BC detection is further limited by healthcare barriers and social stigma, resulting in many patients only presenting with advanced disease in clinics. Studies indicate that 20–30% of women with BC symptoms delay seeking medical care for at least 3 months.[3,17,18] In addition to limited healthcare infrastructure, the medical cost associated with BC diagnosis and treatment is a barrier for many, and prevents timely diagnosis and treatment.[19]

### 1.3. The CoreView imaging on needle (ION) project

In Ghana, treating cancer involves many indirect expenses that are not limited to those incurred during treatment. A recent survey conducted among individuals seeking cancer care in Ghana revealed that only 54.8% of the costs were solely medical, whereas direct non-medical and indirect costs from seeking treatment made up 7.1% and 38.1% of the overall expenses.[20] Such costs included the transportation fees, caregiver fees, and the loss of productivity, deterring patients from seeking necessary

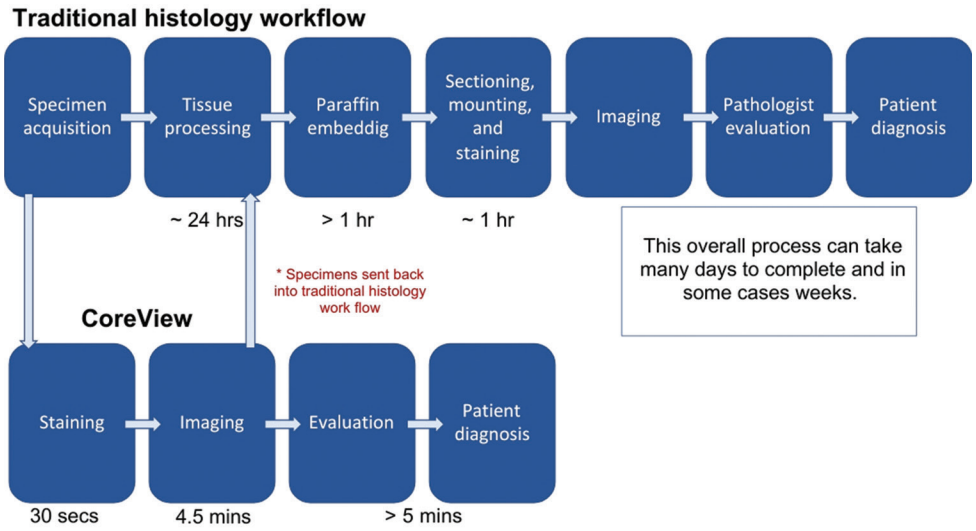


**Figure 1.** Standard histopathological workflow for biopsy processing. The multi-step procedure includes tissue extraction, formalin fixation, paraffin embedding, sectioning, staining, and pathologist evaluation.

follow-up care. As a result, a significant portion of individuals undergoing CNB never receive a definitive diagnosis after biopsy, further hindering effective BC management.[21] One way to mitigate the indirect cost barriers is to introduce lower-cost portable laboratory equipment into rural areas and rural healthcare clinics.

The Human Photonics Laboratory at the University of Washington (UW) has aimed to create a low-cost, portable device that requires minimal electrical needs and training to operate, allowing for rapid, point-of-care diagnosis during a patient's first visit in LMICs and other low-resource areas. The CoreView ION is designed as a cost-effective, accessible imaging solution that produces diagnostic-quality results while minimizing the need for specialized training (Video S1). To overcome the challenges associated with traditional CNB histopathology, the CoreView ION implements manual low-cost components to simplify operation and maintenance, as well as training protocols. By significantly reducing diagnostic turnaround time, this approach has the potential to improve patient outcomes and decrease the number of women in underserved regions who remain undiagnosed due to a multitude of barriers stacked against them.

## 2. Materials and methods

### 2.1. Design and fabrication of the prototype of CoreView ION

To facilitate imaging of CNBs while still on the needle, a prototype fixture was designed, iterated, and tested on animal tissues (Figure 2). Prototype components were modeled using SolidWorks (Dassault Systèmes SolidWorks Corp., USA) and fabricated with a three-dimensional (3D) fused filament fabrication (FFF) printer (Prusa Research a.s., Czech Republic). The CoreView fixture consists of a frame made from structured carbon polycarbonate plates attached to a custom microscope holder. This initial prototype utilized CNB preparation on the needle, employing only drops of fluorescence dye and hand-rinsed saline. Furthermore, future prototypes can integrate CNB staining and rinsing in an automated process (Video S2).

The biopsy is acquired using 14–18-gauge needle biopsy guns and then stained with Rhodamine B and Hoechst. Following staining, the needle biopsy gun is loaded onto a 3D-printed holder. A hand crank is turned to position the specimen against the surface of the fixed, UV-transparent window, which is preset to be the focal plane of the objective lens.

The CoreView ION prototype is equipped with Nikon 4× and 10× objective lens imaging under UV low-powered light-emitting diode (LED) illumination, with multi-axis movement control for both needle biopsy and biopsy compression. The imaging workflow involved staining tissue with Rhodamine B (counterstain, 10 mg/mL) and Hoechst fluorescence dye solutions (Hoechst 33342 nuclear stain, 5 mg/mL), loading the CNB onto the microscope stage, compressing the biopsy surface against a clear quartz coverslip window, and capturing images within 5 min using microscopy with ultraviolet surface excitation (MUSE) technology.[22] Video S2 illustrates the fully automated CoreView ION system, showcasing each integrated component of the final prototype for clear visualization.

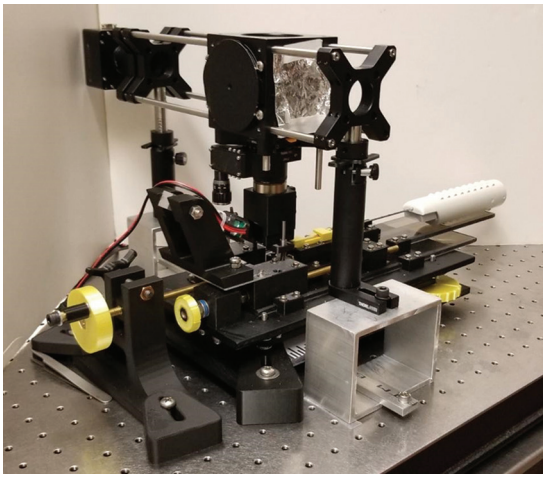

**Figure 2.** The CoreView imaging on needle prototype. The fixture module, modeled in SolidWorks initially and fabricated using a three-dimensional fused filament fabrication printer, features a structured carbon polycarbonate frame and a custom microscope holder for imaging core needle biopsies while still on the needle. The full prototype was designed to be low-powered, low-cost, and compact, allowing for increased portability.

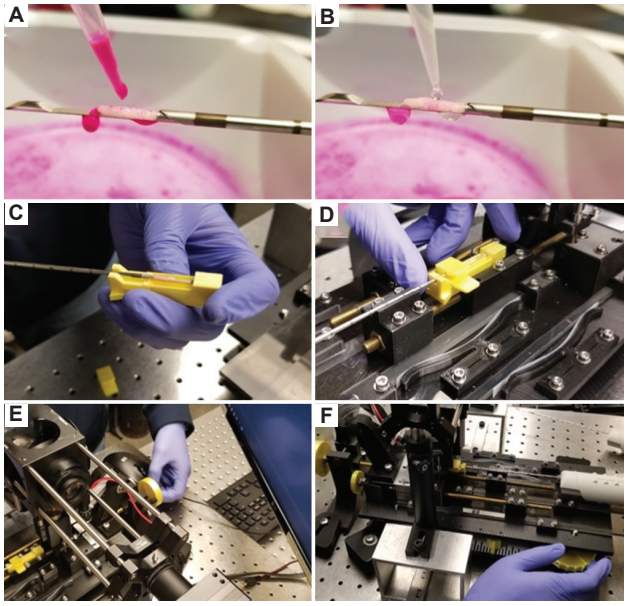


**Figure 3.** CoreView imaging on needle workflow. (A) After the biopsy is acquired, Rhodamine B and Hoechst staining solutions are applied onto the sample, (B) the sample is then rinsed with PBS solution, (C) The biopsy gun is loaded into a three-dimensional-printed holder, (D) the holder is locked into the fixture, (E) a hand crank is used to move the biopsy under the quartz coverslip along the long y-axis, and (F) the biopsy is raised on the z-axis to compress the sample against the coverslip for clear imaging.

### 2.2. Imaging workflow

The current imaging workflow consists of manual staining and loading (1.5 min), MUSE fluorescence imaging while axially scanning the CNB (3 min), and unloading the biopsy (0.5 min), resulting in a total processing time of 5 min from biopsy collection to diagnostic image acquisition (Figure 3). The removal of the CNB from the needle into buffered 10% formalin for conventional downstream processing is the only time the tissue is handled after the core acquisition, which allows for a more pristine surface for MUSE imaging. The MUSE imaging has been shown not to affect conventional hematoxylin and eosin (H&E) imaging of the thin sections taken from the conventional FFPE processing of the CNB.[22]

CNBs were obtained from tissue using a 14-gauge tissue biopsy needle (MC1416 MaxCore, Becton Dickinson/Bard, USA). Following the biopsy procedure, tissues were rinsed with PBS to remove excess debris. A Hoechst and Rhodamine B staining solution was applied until the biopsy top surface was fully wetted. After 30 s, the biopsy was rinsed with PBS to prevent overstaining (Figure 3B). The biopsy needle was then secured in a 3D-printed holder for stability and positioned within the CoreView demonstrator (Figure 3C and D). A hand crank on the left-most end of the demonstrator was used to align the CNB for imaging (Figure 3E). Once aligned, the CNB was brought into contact with a fixed UV-transparent imaging window by adjusting a hand crank, ensuring optimal imaging conditions of a partially flattened CNB surface (Figure 3F).[23] Overhead white lights were turned off, and UV illumination was applied. The images were captured using the Ximea imaging application (XIMEA GmbH, Germany) and Ximea camera (xiD MD091CU-SY, XIMEA GmbH, Germany).

MUSE imaging was performed using 280 nm UV LED light for fluorescence excitation. The Hoechst stain is selectively bound to nuclear material, while Rhodamine B counterstains cytoplasm and surrounding stroma, as well as other structures. Two different objective lenses were utilized for imaging tissue samples. With the 4× objective lens, each biopsy required approximately 10 images to encompass the entire specimen. Images were acquired with an exposure time of 10 s and a 10 dB gain, using 20% overlap for subsequent stitching. With the 10× objective lens (numerical aperture = 0.3), each biopsy required approximately 25 images before stitching, using the same imaging parameters as the 4× objective lens. Images were stitched using ImageJ software (National Institutes of Health, USA).

### 2.3. Compression testing for biopsy integrity

To determine the extent of compression that can be applied to breast CNBs while preserving tissue integrity for downstream histopathological analysis, compression

testing was conducted using both *ex vivo* porcine tissue and a murine tumor model. Fresh CNBs were obtained from *ex vivo* porcine tissue, and the murine tumor FVB/N-Tg (TgMMTV-neu) mouse strain was used as a representative model for human mammary tumors obtained from the Cancer Vaccine Institute in Seattle, Washington, using 14-gauge needles. A total of approximately 20 samples were analyzed for each tissue type across different compression levels.

For porcine tissue, biopsy thickness was measured on the biopsy gun using a caliper before compression. To prevent tissue dehydration, biopsies were showered with PBS solution before being compressed. A screw-based glass-slide compression device was used (Figure 4), consisting of two 3D-printed round disks, each marked with 16 evenly spaced reference points corresponding to a 0.03215 mm increment of compression. The disks were attached to two M3 hex socket screws with a 0.5 mm pitch, ensuring uniform compression across the porcine biopsy specimen. Biopsies from fresh pig breast tissue were compressed to 50%, 40%, and 30% of their original thickness, and calculated using Equation I.

$$\#\text{Marks} = \frac{\text{Original thickness} - \left(\text{Original thickness} \times \%\ \text{compression}\right)}{0.03125\ \text{mm}} \quad \text{(I)}$$

For instance, a 1.2 mm thick biopsy required approximately 23 marks of screw rotation to achieve 40% compression. Table 1 shows this method and the corresponding thicknesses. Two biopsies were collected for each compression condition, and compressed biopsies remained under applied pressure for 2 min before fixation in 10% neutral-buffered formalin. As controls, two additional biopsies were left uncompressed for 2 min before fixation in 10% neutral-buffered formalin for 72 h.

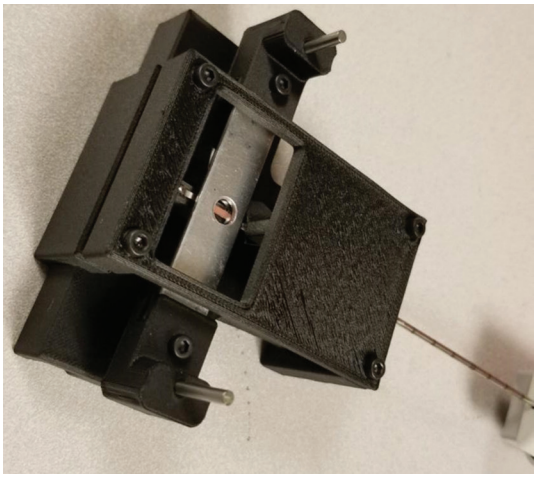

**Figure 4.** Compression testing device. This simple device enables quantitative assessment of tissue deformation while maintaining histopathological integrity.

A calibrated scale was integrated into the CoreView fixture for murine biopsies, enhancing precision in determining biopsy compression levels. This scale was designed based on the average height of a 14-gauge CNB (1.2 mm) and featured black notches spaced at 50 μm increments. In the murine model, biopsies were compressed to 70%, 60%, 50%, and 40% of their original thickness. Before compression, these biopsies were stained with Hoechst and Rhodamine B solutions for 30 s, followed by rinsing with PBS. CNBs were then loaded onto the CoreView demonstrator for controlled compression and imaging using the MUSE microscope. Following imaging, biopsies were fixed in 10% neutral-buffered formalin for 72 h before submission for histological processing.

Following fixation, all specimens were submitted to the UW Histology and Imaging Core for routine H&E staining and imaging. A blinded histopathological evaluation was conducted by a breast pathology specialist, who assessed image sets corresponding to the control and compressed conditions. Each set was evaluated for diagnostic quality and the presence of compression-induced artifacts to determine the effects of controlled compression on biopsy integrity.

### 2.4. Quantification of nuclear edge sharpness using ImageJ

To quantify nuclear edge sharpness, grayscale 10× images of porcine tissue sections imaged using MUSE and conventional H&E brightfield images were analyzed in ImageJ. The scale was set using known reference length of full porcine biopsies (~1 cm in length) and used to calibrate the image scale, spanning a distance of 10,000 μm across 38,702 pixels. Using the Plot Profile tool, intensity values were measured across the diameter of five representative nuclei per imaging modality. For each profile, the minimum and maximum grayscale intensities were recorded, and the 20% and 80% intensity levels from baseline were calculated. The pixel distance between these two points was used as a quantitative measure of how sharply intensity changed at the nuclear boundary. Average distances were computed for each modality to compare edge gradients between MUSE and H&E images.

**Table 1. Biopsy thickness under compression**

| Percentage of compression | Original thickness | Target thickness | Marks turned |
|---|---|---|---|
| 50 | 1.22 mm, 1.19 mm | 0.61 mm, 0.60 mm | 19.5, 19.2 |
| 40 | 1.12 mm, 1.22 mm | 0.45 mm, 0.49 mm | 21.4, 23.4 |
| 30 | 1.17 mm, 1.30 mm | 0.35 mm, 0.39 mm | 26.2, 29.1 |

## 3. Results and discussion

### 3.1. MUSE imaging of porcine tissues

Using the CoreView prototype, we successfully imaged fresh pig breast tissue within 5 min after biopsy acquisition, well within the 1–2 h post-ischemic time target for tissue specimens before formalin fixation. The rapid imaging workflow demonstrated the potential for near-real-time evaluation of tissue morphology, a crucial factor in point-of-care applications. The resulting panoramic images exhibited preservation of cellular architecture, with well-defined nuclear contrast and strong contrast between nuclei and the surrounding stromal components (Figure 5). While these findings are promising, it is important to note that no human tissues were used in this study; further validation with human biopsy samples will be necessary to assess clinical applicability and ensure translational relevance.

Despite the clarity of nuclear features, challenges were observed in capturing detailed imaging of ductal structures within the pig breast tissues. This limitation may be attributed to differences in glandular composition between porcine and human breast tissue, to variations in tissue density and properties that influence optical penetration and contrast, and to the ability of the needle biopsy gun to sample targeted areas.

### 3.2. MUSE imaging of murine tumor models

Mouse tumor samples from the FVB/N-Tg (TgMMTV-neu) mouse strain, provided by the Cancer Vaccine Institute in Seattle, WA, were utilized as additional specimens. The tumor images were captured using the CoreView prototype after biopsy acquisition and staining (Figure 6).

The resulting images demonstrated the system's capability to visualize cancerous specimens, which exhibited distinctly different density and tissue properties compared to the pig breast tissues previously tested. While the images provided valuable feedback on the device's ability to assess diseased tissue, overall image clarity was lower than that observed in pig breast tissue. Notably, nuclear features in the murine samples appeared with limited structural detail, and overall tissue architecture was poorly defined. Several factors may contribute to the reduced image clarity observed in the murine tumor sample. First, inconsistencies in later quartz coverslip cleaning likely introduced optical artifacts, such as blurring. In addition, as these tumors were obtained as residual specimens, the tissue had been acquired a considerable period before imaging and had experienced 6 h of ischemic time, resulting in tissue degradation and loss of structural integrity. Furthermore, the staining protocol using Rhodamine B and Hoechst may have influenced the image brightness and contrast, potentially obscuring finer morphological details. Future studies will aim to refine tissue preparation protocols and optimize staining conditions to improve imaging quality and consistency across different tissue types.

Despite the suboptimal results observed in the murine tumor samples, high-quality MUSE images have been successfully obtained from core biopsies in non-needle-based

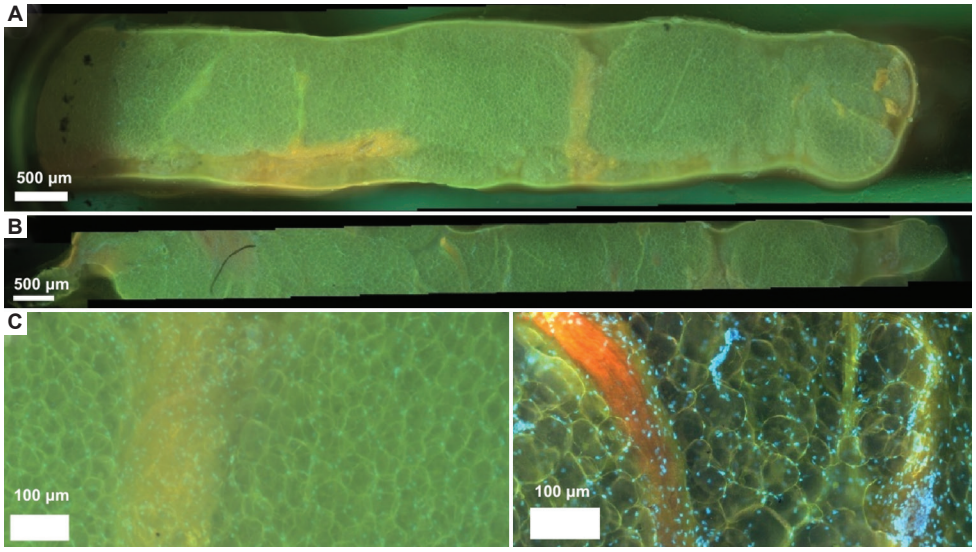


**Figure 5.** Microscopy with ultraviolet surface excitation imaging of fresh porcine breast tissue obtained via 14-gauge core needle biopsy gun. (A) Pig breast sample imaged using a 4× objective lens, stitched with ImageJ. Scale bar: 500 µm; magnification: 10×, (B) Pig breast sample imaged using a 10× objective lens, stitched with ImageJ. Nuclei are stained with Hoechst and appear blue/teal compared to the Rhodamine B counterstain. Scale bar: 500 µm; magnification: 10x, (C) Zoomed-in 4× MUSE image of pig breast tissue as seen in (A). Scale bar: 100 µm; magnification: 4×, (D) Zoomed-in 10× MUSE image of pig breast tissue as seen in (B). Scale bar: 100 µm; magnification: 4×.

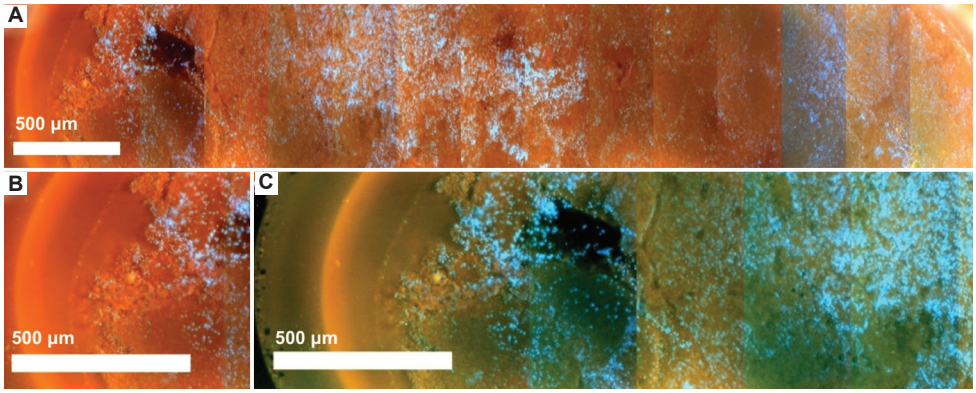


**Figure 6.** Microscopy with ultraviolet surface excitation imaging of a mouse tumor model obtained via a 14-gauge core needle biopsy gun. (A) Image of the mouse tumor, stitched using ImageJ. Scale bar: 500 µm; magnification: 10×, (B) Zoomed-in segment of the tumor from (A), stitched using ImageJ. Nuclei are stained with Hoechst with a Rhodamine B counterstain. Scale bar: 500 µm; magnification: 10×, (C) Color-adjusted image using ImageJ to improve visual comparison between pig breast biopsies and mouse tumor biopsies. Scale bar: 500 µm; magnification: 10×.

applications (Figure 7A and B). These comparative images demonstrate the level of image clarity achievable with optimized sample handling and preparation, further supporting the notion that, with continued refinement, the CoreView ION platform holds strong promise for rapid, point-of-care diagnostic applications.

### 3.3. Biopsy integrity under compression testing

Understanding the effects of compression on biopsy samples is critical since the prototype requires a flat surface for planar microscopic imaging. When the biopsy is pressed against the imaging window, the specimen is flattened, allowing for a greater percentage of the tissue surface in full focus and direct contact with the quartz coverslip, improving image quality and resolution. However, CNBs are structurally fragile, and external stressors can lead to mechanical breakdown. Therefore, confirming if compression leads to tissue damage that could compromise downstream histopathological analysis is essential.

After conducting a study to assess the effects of varying compression levels on biopsy integrity, the pig breast samples were processed through a standard histopathological workflow at the UW Histology and Imaging Core for H&E staining (Figure 8). The resulting digital pathology slides were reviewed by a breast pathology specialist from the UW Medicine Department of Pathology. On evaluation, the pathologist remarked, "The tissue quality and staining are excellent… able to make a diagnosis on tissue samples of this quality" (Dr. Suzanne Dintzis, MD, PhD, October 28, 2022). These findings, later confirmed by a board-certified pathologist from the University of California, Davis, Department of Pathology, indicate that even under high compression—flattening the specimen to 30% of its original thickness, more than the

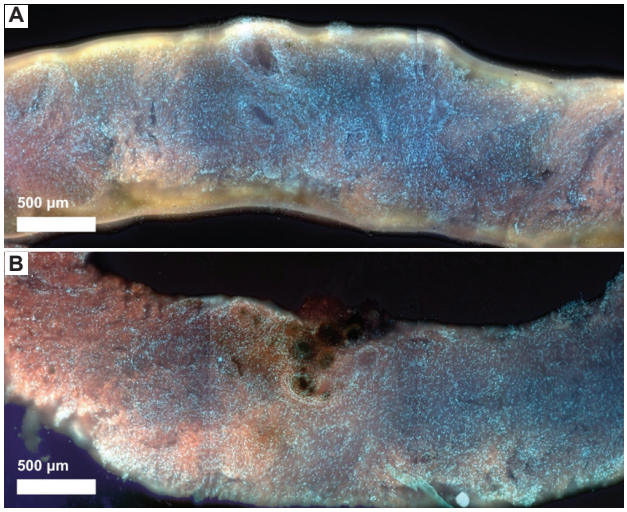


**Figure 7.** High-quality MUSE images from core biopsies in non-needle-based applications. (A) Fresh normal prostate core biopsy stained with alternative Rhodamine and Hoechst, imaged using MUSE by the Levenson Lab, University of California, Davis. Scale bar: 500 µm; magnification: 10×, (B) Fresh cancerous prostate core biopsy stained with alternative Rhodamine and Hoechst, also imaged using MUSE by the Levenson Lab, University of California, Davis.[22] Scale bar: 500 µm; magnification: 10×.
Abbreviation: MUSE: Microscopy with ultraviolet surface excitation.

compression applied using the CoreView ION system—the tissue maintained structural and molecular integrity. This preservation suggests that the samples will remain viable for downstream pathological assessment.

It is important to note that while the compression mechanism utilized in this study differs from that of CoreView ION, the employed test fixture allowed for more precise compression control, as well as improved specimen accessibility and processing speed. One limitation of this study is the absence of cancerous tissue in the test samples; while healthy tissue demonstrated no observable differences across

compression levels, diseased tissue may respond differently. Further studies incorporating malignant samples are necessary to evaluate potential compression-induced artifacts.

Murine tissue sections were also submitted to the UW HIC for H&E staining and subsequent pathological evaluation (Figure 9). The digital slides were reviewed by a breast pathologist, who similarly observed that compression did not appear to compromise image integrity or impede accurate diagnosis. However, these samples consisted of spontaneous mammary tumors in mice, necessitating additional validation using human breast tissue to rule out the possibility of compression-induced artifacts. Murine pathology differs significantly from human pathology due to inherent structural variations, including a greater density of hair follicles, differences in stromal composition, and variation in glandular architecture. These distinctions underscore the importance of follow-up studies in human tissue to ensure the translatability of findings to clinical practice.

### 3.4. CoreView ION prototype performance

Grayscale 10× porcine tissue images acquired using MUSE and H&E staining were compared to assess nuclear contrast and edge sharpness. In the MUSE image, nuclei appear brighter than the surrounding stroma, whereas in the H&E image, nuclei are darker. Intensity profiles across representative nuclei demonstrate this inverse contrast pattern, with the MUSE signal increasing in nuclear regions and the H&E signal decreasing due to the dark hematoxylin stain. Relative quantitative analysis showed that the average pixel distances, used as a measure of nuclear edge sharpness, ranged from 20% to 80% of the normalized intensity range, were 10 pixels in MUSE images and 8.2 pixels in H&E images. This indicates that MUSE provides positive nuclear contrast with a gradual transition at nuclear boundaries compared to the steeper edge seen in H&E-stained sections (Figure 10 and Table 2). Notably, our UV dose was approximately 20 times lower than that used in previous MUSE studies. We employed unfocused illumination with longer camera dwell times to reduce light intensity and minimize photobleaching.[24]

The CoreView ION fixture demonstrates the capability to generate diagnostic-quality images within a remarkably short timeframe, producing a complete image within 5 min with no failures. By remaining on the biopsy needle,

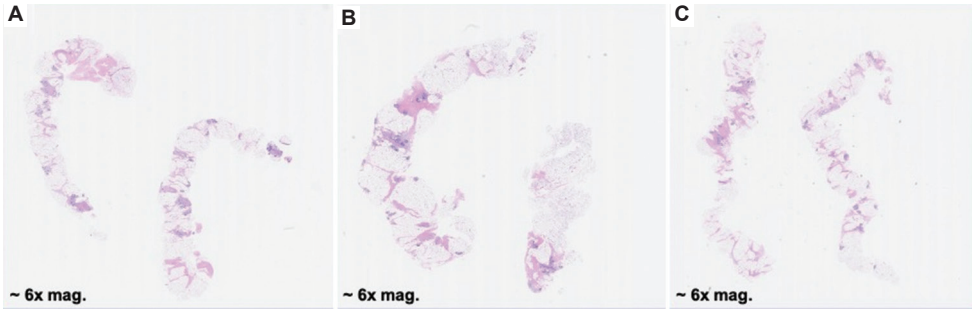


**Figure 8.** H&E scans of pig breast biopsies. (A) H&E slide of non-diseased porcine breast biopsy compressed to 50% of original thickness, showing no compression artifacts. Magnification: 6×, (B) H&E slide of non-diseased porcine breast biopsy compressed to 40% of original thickness, showing no compression artifacts. Magnification: 6×, (C) H&E slide of non-diseased porcine breast biopsy compressed to 30% of original thickness, showing no compression artifacts. Magnification: 6×.
Abbreviation: H&E: Hematoxylin and eosin.

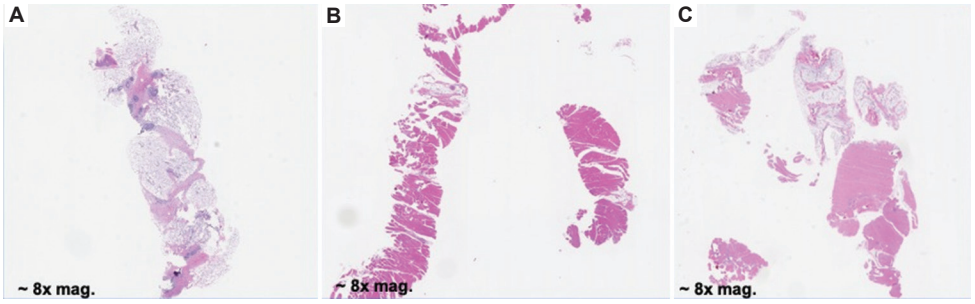


**Figure 9.** H&E scans of mouse tumor biopsies. (A) Murine tissue compressed to 70% of its original thickness. Magnification: 8×, (B) Murine tissue compressed to 60% of its original thickness. Tissue artifacts occurred during histology processing, resulting in a fragmented sample. Magnification: 8×, (C) Murine tissue compressed to 50% of its original thickness. Tissue artifacts occurred during histology processing, resulting in a fragmented sample. Magnification: 8×.
Abbreviation: H&E: Hematoxylin and eosin.

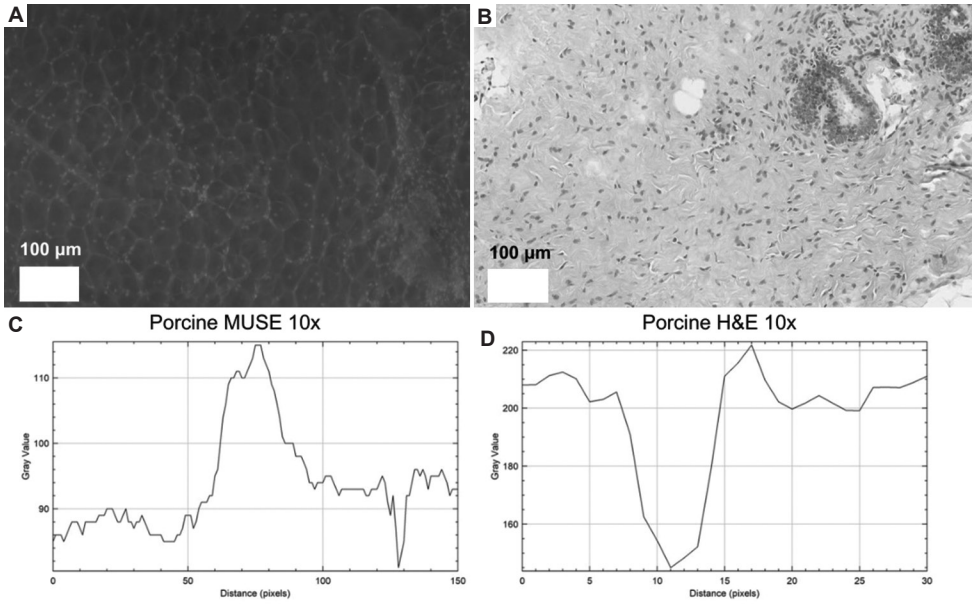


**Figure 10.** Grayscale MUSE and H&E porcine images with resolution analysis. (A) Grayscale porcine MUSE image. Scale bar: 100 µm; magnification: 10×, (B) Grayscale porcine H&E image, shown for resolution comparison. Scale bar: 100 µm; magnification: 10×, (C) Intensity profiles across representative nuclei of (A); (D) Intensity profiles across representative nuclei of (B).
Abbreviations: H&E: Hematoxylin and eosin; MUSE: Microscopy with ultraviolet surface excitation.

**Table 2. Nuclear intensity measurements from 10×porcine tissue images acquired with MUSE and from H and E brightfield**

| Nuclei and imaging modality | Minimum intensity | Maximum intensity | 20% from baseline | 80% from baseline | Pixel distance |
|---|---|---|---|---|---|
| MUSE 10 × Pig Nucleus 1 | 86.00 | 115.00 | 91.80 | 109.20 | 10.0 |
| MUSE 10 × Pig Nucleus 2 | 86.50 | 111.00 | 91.50 | 109.20 | 11.0 |
| MUSE 10 × Pig Nucleus 3 | 81.87 | 112.90 | 88.08 | 106.70 | 8.0 |
| MUSE 10 × Pig Nucleus 4 | 97.00 | 124.09 | 102.40 | 118.70 | 9.0 |
| MUSE 10 × Pig Nucleus 5 | 92.97 | 122.72 | 98.92 | 116.80 | 12.0 |
| Average MUSE 10 × Pig | | | | | 10.0 |
| H&E 10 × Pig Nucleus 1 | 145.00 | 212.40 | 198.92 | 158.48 | 6.0 |
| H&E 10 × Pig Nucleus 2 | 91.00 | 221.20 | 195.16 | 117.04 | 12.0 |
| H&E 10 × Pig Nucleus 3 | 97.00 | 207.00 | 185.00 | 119.00 | 6.0 |
| H&E 10 × Pig Nucleus 4 | 115.90 | 240.00 | 215.20 | 140.70 | 7.0 |
| H&E 10 × Pig Nucleus 5 | 128.00 | 212.00 | 195.20 | 144.80 | 10.0 |
| Average H and E 10 × Pig | | | | | 8.2 |

Abbreviations: H&E: Hematoxylin and eosin; MUSE: Microscopy with ultraviolet surface excitation.

the biopsy stayed intact until the compression step in the imaging process. When an earlier version of CoreView used a fluidic lab-on-chip approach without compression, the removal process recovered only 90% intact CNBs for fresh breast tissue, necessitating the CoreView ION approach.[25] This efficiency is a key advantage for rapid diagnostic workflows, allowing for near-instantaneous feedback during pathology assessments and CNB procedures. The prototype's ability to produce high-quality digital images at low magnification shows its potential as a viable alternative to conventional histopathology techniques (Figure 1). The images maintain diagnostic integrity, enabling pathologists to analyze tissue samples effectively without the need for traditional histological processing steps.

Furthermore, image compression was evaluated to determine its impact on downstream histopathological analysis, including tests with both pig and mouse tissue samples. Results indicated that compression does not compromise the integrity of downstream histopathological analysis, and the samples remain viable for pathological

evaluation in a standard workflow. Compression allowed for sharper image quality across the length of the CNB and expanded the area being imaged by up to two times the original area.

A significant strength of the system lies in its minimal requirements for electrical power. The CoreView prototype operates with only three components requiring electrical power (LED: 1.5W, Ximea camera: 3.0W, and a computer, which can be a battery-powered laptop), showing promising proof-of-concept work for low-cost and accessible solutions for rural and low-resource clinical settings. With a total cost of goods of less than USD 8,000 (excluding labor), including a camera costing USD 4,000, the system offers an affordable option compared to existing digital pathology solutions. While the CoreView ION has not been fully automated, this was found unnecessary for achieving rapid imaging and analysis, specifically for the stain protocol. The simplicity and speed of the system suggest that automation could easily be implemented in future iterations, but even in its current form, the workflow remains efficient and practical. If further automation and higher-powered LEDs were implemented, the 5-min process could be even faster while minimizing errors. With increased speed provided by system automation, a 20× objective could be implemented at an incrementally higher cost.

### 3.5. System limitations and challenges

While the CoreView prototype offers a promising proof-of-concept for rapid and low-cost imaging of CNBs in BC diagnostics, several limitations must be considered before clinical implementation. One major limitation is the expectation that a single core is sufficient for a diagnosis. If a second core is needed, then the needle would necessitate a thorough cleaning and rinsing protocol, introducing potential workflow inefficiencies and requiring further validation for sterility. An example of global use of a reusable CNB device (Figure 11), which could be incorporated into a CoreView ION imaging workflow, with multiple clean needles being used with one reusable biopsy gun.

Another issue with the system is the dependence on quartz coverslips, which are significantly more expensive than standard glass slides. This cost factor may present a barrier to widespread adoption, particularly in low-resource, rural settings where affordability is a primary concern. Between each sample, the quartz glass required cleaning or replacing if broken, leading to workflow inefficiency between samples.

In addition, the imaging workflow and staining process remain unoptimized. The current staining and imaging parameters were developed as proof-of-concept and have not yet been refined for clinical-grade imaging. Further optimization is necessary to enhance contrast, reduce imaging artifacts, and improve overall diagnostic quality. Furthermore, the study has not yet demonstrated high-quality imaging of malignant BC human tissues. The initial results provide a foundation for future work, but additional validation using a diverse range of cancerous tissues is required to assess the system's true diagnostic potential. These limitations highlight areas for future improvement, including optimization of the staining and imaging workflow, cost reduction strategies, and expanded validation studies to ensure clinical applicability with and without artificial intelligence (AI) enhanced diagnosis from the resulting CoreView ION images.

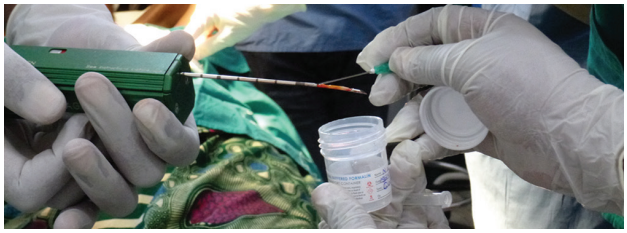

**Figure 11.** Reusable BARD coring needle handpiece and low-cost disposable needle are displayed to the photographer during a training course in Rwanda on breast biopsy procedure for palpable breast masses. The photograph is provided by Dr. Jane Brock, formerly at Brigham and Women's Hospital, Harvard University.

### 3.6. Future improvements and optimization

As the CoreView ION is an initial proof-of-concept prototype, there are potential directions for further refinement of the imaging strategy and design. At present, the prototype depends on a computer system for MUSE imaging, necessitating access to electrical power and a computer connection. However, recent studies have demonstrated the feasibility of utilizing MUSE imaging through smartphones.[24] The Pocket MUSE system, which employs an optical module attached to the rear lens of a smartphone, facilitates high-quality fluorescence imaging at a significantly reduced cost. Incorporating Pocket MUSE technology and concepts into the CoreView ION could eliminate the requirement for a computer connection, enhancing its usability in rural settings. Furthermore, the existing low-powered UV LEDs in the current fixture could potentially be replaced with a battery-powered module, allowing the system to operate solely on battery power.

The current prototype relies on a clean transparent coverslip, which introduces workflow inefficiencies and cleaning challenges. Recent advancements in imaging technologies, such as fluorescence-imitating brightfield imaging (FIBI), have demonstrated the capability to capture tissue images without coverslip compression.[26,27]

Eliminating the need for a clean glass surface would streamline the imaging process. Integrating FIBI thick tissue imaging, which can produce a 30× greater signal-to-noise ratio compared to MUSE images, can accelerate the imaging process and eliminate UV optical hazards, as well as the need for expensive quartz glass. Although the extended depth of focus algorithm would eliminate the need for tissue compression, its deployment requires fast axial scanning, which would increase cost and complexity for the future portable CoreView ION system. While these technological adaptations are promising for low-resource and point-of-care applications, clinical translation will ultimately require validation using human biopsy specimens to confirm clinical diagnostic accuracy and performance. Expanding accessibility and accelerating diagnosis with AI-enhanced analysis of thick tissue biopsy images are within reach, as these pre-clinical results suggest that the CoreView instrument can provide rapid, point-of-care diagnosis for the most prevalent cancers in Africa: BC, cervical cancer, and prostate cancer.[28-32]

## 4. Conclusion

The CoreView ION system demonstrates the feasibility of rapid, on-needle imaging for CNB analysis, offering a potential low-cost point-of-care solution for BC diagnostics. By integrating MUSE imaging with a more streamlined workflow, this system enables bedside visualization of biopsy samples, compared to the days to months of delays experienced now in low-resource settings. Further optimization and clinical validation with human tissue will be necessary to fully establish its role in improving BC survival rates by providing greater access to these new rapid diagnostic pathways. This approach is a significant advancement in pathology, using thick tissue biopsy imaging (MUSE and FIBI), as on-needle imaging has not been previously explored. By enabling real-time evaluation at the point of care, the CoreView ION system has the potential to greatly reduce patient burden, expedite treatment decisions, and ultimately improve clinical outcomes in BC diagnostics.

## Supplemental information

**Video S1.** Video demonstration of the CoreView imaging on needle concept. The objective lens is focused on the top surface of the compressed tissue that rests on the coring needle. Only the contacting parts (mechanical extension of the objective lens and the small specimen chamber) are shown in cross-section.

**Video S2.** Video demonstration of CoreView imaging on needle workflow with animated computer-aided design drawings.

## Acknowledgments

We extend our gratitude to Dr. Farzad Fereidouni at the University of California, Davis, for his pioneering work in MUSE imaging and his invaluable contributions to the development of the CoreView ION system. Dr. Jane Brock provided the photograph taken of the CNB procedure using a reusable biopsy gun. We appreciate the input of Dr. Beatrice Wiafe Addai for insightful discussions on the global needs for rapid BC diagnosis at the bedside and the unmet need for a low-cost portable CoreView system for more rural healthcare clinics in West Africa.

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## Conflict of interest

Matthew D. Carson and Eric J. Seibel are co-authors of a patent owned by the UW. They are also the participants in the royalty-sharing program.

## Author contributions

*Conceptualization*: Eric J. Seibel, Matthew D. Carson
*Investigation*: Jocelyn R. Jensen, Duy Do, Yuan-ping Chang, Suzanne Dintzis, Richard M. Levenson
*Methodology:* All authors
*Writing–original draft*: Jocelyn R. Jensen
*Writing–review & editing:* Eric J. Seibel, Matthew D. Carson, Suzanne Dintzis, Richard M. Levenson

## Ethics approval and consent to participate

De-identified human prostate tissue images were provided by the University of California, Davis, under Institutional Review Board exemption FWA No: 00004557, granted on June 23, 2023. The exemption was issued under the supervision of Principal Investigator Dr. Richard Levenson. As all samples were de-identified before transfer, informed consent was not required in accordance with institutional and federal guidelines.

## Consent for publication

Not applicable.

## Availability of data

Data used in this work are available from the corresponding author on reasonable request.

## Further disclosure

The CoreView ION prototype was first presented by Dr. Eric Seibel at the 2022 NCI IMAT Principal Investigators Meeting in Lawrence, Kansas, on December 1, 2022, in a presentation titled "Rapid Needle Biopsy Assessment at Point of Care to Advance Personalized Cancer Therapy."

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LETTER TO EDITOR

# Mass balance and energy balance in body weight regulation: A response to Theodorakis' comments

**Anssi H. Manninen***

Department of Research and Development, Dominus Nutrition Oy, Northern Ostrobothnia, Finland

Dear Editor,

I wish to begin by expressing my sincere gratitude to Dr. Theodorakis for his interest in my recent article[1] and for taking the time to engage with its content through his letter to the editor.[2] Such scholarly dialog is invaluable in advancing our collective understanding of complex topics like body weight regulation.

My article aims to introduce the core ideas of the mass balance model, a novel paradigm for understanding body weight dynamics initially proposed by my esteemed colleague, Dr. Arencibia-Albite.[3,4] Given that my work builds directly upon this foundation, I strongly encourage readers – particularly those intrigued by this approach – to consult Dr. Arencibia-Albite's comprehensive publications. These articles provide a detailed exploration of the model's intricacies, offering the depth and rigor necessary to fully appreciate its theoretical and practical implications.

However, I must address a misinterpretation raised by Dr. Theodorakis, who suggests that my research claims "energy balance has no impact on body mass," calling this view "scientifically inaccurate."[2(p142)] I respectfully note that this does not accurately reflect the position presented in my paper. Rather than dismissing the role of energy balance, the mass balance model integrates and builds upon it by situating it within a broader context of mass dynamics. My article does not deny the role of energy intake and expenditure in influencing body mass. Instead, it posits that these energy-related processes ultimately exert their effects through corresponding changes in mass input and output, as articulated by the mass balance framework. This perspective complements – rather than contradicts – the energy balance theory, positioning mass balance as a critical lens through which energy-related processes can be more accurately understood in the context of weight stability and change.

To clarify further, Dr. Arencibia-Albite's study demonstrates that weight stability can coexist with a persistent energy imbalance – challenging the traditional assumption that energy balance is the sole determinant of steady body weight. My study builds on this insight, emphasizing that mass balance provides a unifying mechanism to explain such phenomena. The synergy between our contributions underscores the importance of reading both works together to fully appreciate the scope of the mass balance paradigm.

Finally, I am pleased to share that Dr. Arencibia-Albite is preparing a forthcoming publication that will further refine the mass balance model and address common misconceptions about its application. This upcoming work is expected to provide additional clarity and resolve lingering questions, and I look forward to its contribution to the ongoing discourse.

In closing, I appreciate Dr. Theodorakis' engagement and welcome the opportunity for continued discussion on this topic. The complementary nature of my study and

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Dr. Arencibia-Albite's foundational work highlights the potential for collaborative exploration to deepen our understanding of body weight regulation – an endeavor I hope will inspire further research and refinement.

## Conflict of interest

The author declares no conflict of interest.

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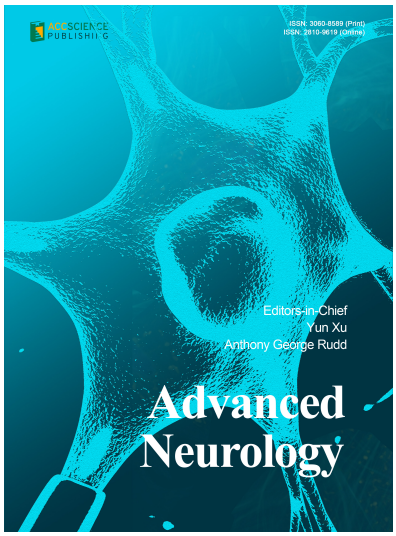


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