

REVIEW ARTICLE

Engineering extracellular matrix-mimetic recombinant proteins for stem cell niche and clinical translation

Jun-Hyeog Jang* 

Department of Biochemistry, Inha University School of Medicine, Incheon, Republic of Korea

Abstract

The extracellular matrix (ECM) constitutes a highly dynamic and biochemically complex three-dimensional microenvironment that governs stem cell behavior through a constellation of biophysical and biochemical signals. Recombinant protein engineering has emerged as a transformative strategy to recapitulate the functional architecture of native ECM, enabling the design of defined, scalable, and clinically translatable biomaterials for stem cell niche engineering. This review comprehensively examines the molecular biology of key ECM components—including fibronectin, laminin, collagen, elastin, and fibrillin—and their critical roles in modulating stem cell self-renewal, directed differentiation, and protection from cellular senescence. This review discusses state-of-the-art protein engineering approaches encompassing domain engineering, elastin-like polypeptide (ELP) fusion strategies, chimeric multi-domain architectures, and site-specific functionalization. The applications of ECM-mimetic recombinant proteins across multiple biomaterial platforms—electrospun scaffolds, hydrogels, implant surface coatings, and nanoparticle delivery systems—are systematically reviewed. Finally, the translational challenges of manufacturing scalability, immunogenicity, and regulatory pathways are addressed, and a forward-looking perspective is offered on the convergence of synthetic biology, computational protein design, and advanced manufacturing to accelerate the clinical translation of ECM-mimetic therapeutics.

***Corresponding author:**Jun-Hyeog Jang
(jujang@inha.ac.kr)

Citation: Jang J-H. Engineering extracellular matrix-mimetic recombinant proteins for stem cell niche and clinical translation. *Global Transl Med*. doi: 10.36922/GTM026210021

Received: May 20, 2026**Revised:** July 17, 2026**Accepted:** September 11, 2026**Published online:** September 28, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Keywords: Extracellular matrix; Recombinant proteins; Stem cell niche; Biomaterials; Elastin-like polypeptide; Protein engineering; Clinical translation

1. Introduction

Stem cells occupy specialized anatomical niches that tightly regulate their fate decisions through an intricate interplay of soluble signaling molecules, cell-cell contacts, and biophysical cues embedded within the extracellular matrix (ECM). The ECM is far more than a passive structural scaffold; it serves as a dynamic information reservoir that stores, presents, and releases growth factors while transducing mechanical signals through transmembrane integrin receptors into the intracellular signaling cascades that ultimately govern self-renewal versus differentiation¹⁻⁴. Dysregulation of ECM composition and mechanics underlies numerous pathological conditions, including organ fibrosis, cancer progression, and the age-related decline in stem cell function, underscoring the therapeutic importance of restoring ECM homeostasis.^{5,6}

Despite the biological importance of the ECM, recapitulating its complexity *in vitro* and *in vivo* has historically relied on ill-defined biological matrices such as Matrigel or decellularized tissue extracts. These materials, while biologically rich, suffer from critical limitations including batch-to-batch variability, xenogeneic contamination, risk of pathogen transmission, undefined composition, and the impossibility of independent variable control in mechanistic studies.^{7,8} These shortcomings present formidable obstacles to clinical translation, where regulatory agencies demand well-characterized, reproducible, and safe biomaterials.

Recombinant protein engineering offers a compelling solution to these challenges by enabling the design and production of structurally defined ECM-mimetic proteins with precisely controlled biochemical and biophysical properties.^{9,10} Through the tools of molecular cloning, synthetic gene design, directed evolution, and heterologous expression systems, it is now possible to produce functional analogs of native ECM proteins at scale, incorporating specific bioactive domains, tunable mechanical properties, and orthogonal conjugation handles for downstream functionalization.¹¹ These engineered proteins can be incorporated into diverse biomaterial platforms—from electrospun nanofiber scaffolds to injectable hydrogels and implant surface coatings—thereby enabling the construction of truly defined stem cell niches.

This review synthesizes the current state of knowledge on ECM-mimetic recombinant protein engineering and its applications in stem cell niche construction and clinical translation. This review first describes the molecular biology of key ECM components and their functional roles in stem cell regulation. Protein engineering strategies are then systematically reviewed with emphasis on domain engineering, elastin-like polypeptide (ELP) fusion technologies, and chimeric multi-domain protein design. The integration of these proteins into biomaterial platforms is examined, followed by a critical analysis of the challenges and opportunities for clinical translation. Throughout, design principles derived from foundational and recent research are highlighted, charting a course toward the next generation of defined, clinically translatable ECM-mimetic therapeutics.

1.1. Literature search strategy

The literature discussed in this review was identified through a structured search of the PubMed/MEDLINE, Scopus, Web of Science Core Collection, and Google Scholar databases, covering publications from January 2000 to June 2026. The search combined controlled-vocabulary and free-text terms using Boolean operators,

including (“extracellular matrix” OR “ECM”) AND (“recombinant protein” OR “protein engineering”) AND (“stem cell” OR “niche” OR “tissue engineering”), together with component-specific terms (“fibronectin,” “laminin,” “collagen,” “elastin-like polypeptide,” “fibrillin”) and application-specific terms (“hydrogel,” “electrospinning,” “bioprinting,” “clinical translation,” “GMP”). Priority was given to peer-reviewed original research and authoritative reviews, with particular attention to seminal foundational studies and to recent advances published between 2020 and 2026. The reference lists of retrieved articles were additionally screened by hand to capture relevant sources not returned by the database queries. Non-English articles, conference abstracts without an accompanying full text, and studies lacking mechanistic or translational relevance to ECM-mimetic protein engineering were excluded. Given the breadth of the field, this article is written as a critical narrative synthesis that prioritizes conceptual and translational significance rather than an exhaustive systematic enumeration of the literature.

2. Key ECM components and their roles in the stem cell niche

An integrated overview of the five ECM proteins discussed in this section—together with their principal functional domains, the stem cell receptors they engage, and the downstream signalling pathways they control—is presented schematically in [Figure 1](#) and summarized in [Table 1](#). A focused comparison of their relative biological functions, engineering challenges, and application suitability is developed in [section 2.6](#) and [Table 2](#).

2.1. Fibronectin

Fibronectin (FN) is a high-molecular-weight glycoprotein (~220 kDa per monomer) that exists as a disulfide-linked dimer and is one of the most extensively characterized ECM adhesion molecules. The FN molecule is composed of three types of repeating structural modules—FNI, FNII, and FNIII—organized into functional domains that mediate binding to cells, collagen, heparin, fibrin, and other ECM molecules.¹² The central cell-binding domain, which includes the FNIII9-10 module housing the canonical RGD (Arg-Gly-Asp) tripeptide and synergistic PHSRN sequence, is the primary mediator of integrin $\alpha 5 \beta 1$ -dependent cell adhesion and downstream mechanosensing.^{13,14}

In the context of stem cell biology, fibronectin plays multifaceted roles that extend beyond mere adhesion. FN-integrin signaling activates the FAK/Src/PI3K and MEK/ERK pathways, which intersect with pluripotency networks including Oct4, Sox2, and Nanog in embryonic

Table 1. Key ECM proteins, their functional domains, stem cell regulatory roles, and recombinant engineering applications

ECM protein	Key domains	Stem cell function	Recombinant form	Application
Fibronectin	RGD, FNIII9-10, heparin-binding	Adhesion, osteogenesis, stemness, anti-senescence	FNIII9-10, FN9-10ELP fusions	Scaffold coating, surface functionalization
Laminin $\alpha 2$	LG1-3 domains	Stemness maintenance, neural niche	Recombinant LG1-3	MSC culture substrate
Laminin $\alpha 5$	LG1-3 domains	Self-renewal, stemness maintenance	Recombinant $\alpha 5$ LG1-3 domains	MSC culture substrate
Collagen I	RGD, triple helix	Proliferation, differentiation	Collagen hydrogel + FGF2	3D MSC culture, bone
Elastin/ELP	VPGXG repeats	Mechanical compliance, stemness	ELP fusion constructs	Injectable hydrogels
Fibrillin	Calcium EGF, TB domains	TGF- β regulation, niche structure	Chimeric fibrillin-elastin	Vascular/MS niche

Abbreviations: ECM: Extracellular matrix; ELP: Elastin-like polypeptide; FGF2: Fibroblast growth factor 2; MSC: Mesenchymal stem cell; TGF- β : Transforming growth factor beta.

Table 2. Comparative assessment of recombinant ECM proteins: relative biological functions, engineering challenges, and application suitability.

ECM protein	Dominant biological function	Structural complexity/ preferred expression host	Key engineering challenge	Best-suited application
Fibronectin (FNIII9-10)	Integrin $\alpha 5 \beta 1$ adhesion, mechanosensing, growth-factor sequestration	Low; disulfide-free β -sandwich, expressed in <i>E. coli</i>	Preserving bioactive orientation on surfaces	Universal adhesion/osteogenic/anti-senescence coatings
Laminin (LG1-3)	Tissue-specific basement-membrane niche signalling	High; heterotrimeric, benefits from mammalian glycosylation	Trimer assembly and glycosylation-dependent activity	Pluripotent/neural/hematopoietic niche substrates
Collagen I	Mechanical scaffolding, fibrillar architecture	High; triple helix requires prolyl hydroxylation	Fully recombinant triple-helix stability	Hybrid hydrogels for 3D culture and bone
Elastin/ELP	Tunable, stimulus-responsive mechanics	Low; repetitive VPGXG, expressed in <i>E. coli</i>	Limited intrinsic receptor specificity; <i>in vivo</i> persistence	Injectable/thermoreponsive carriers and hydrogels
Fibrillin	TGF- β /BMP bioavailability and niche homeostasis	Very high; cysteine-rich, calcium-dependent domains	Oxidative folding of disulfide-rich domains	Perivascular/skeletal growth-factor-regulated niches

Abbreviations: BMP: Bone morphogenetic protein; ECM: Extracellular matrix; ELP: Elastin-like polypeptide; TGF- β : Transforming growth factor beta.

stem cells, and direct osteogenic, chondrogenic, and neurogenic lineage commitment in mesenchymal stem cells (MSCs).¹⁵ The heparin-binding domain of FN (FNIII12-14) is particularly important for sequestering heparin-binding growth factors such as FGF-2 and VEGF within the pericellular space, creating local morphogen gradients that powerfully shape stem cell fate.¹⁶ Recombinant FNIII9-10 and chimeric FN-elastin constructs have been extensively validated as defined substrates for MSC culture, demonstrating superiority over adsorbed native FN in promoting sustained stemness and targeted differentiation.¹⁷

2.2. Laminin

Laminins constitute a family of heterotrimeric glycoproteins assembled from α , β , and γ chains, with at least 15 distinct isoforms exhibiting tissue-specific expression patterns. The α chain, of which five variants ($\alpha 1$ – $\alpha 5$) have been

identified, contains the globular LG1–5 domains at the C-terminus that mediate high-affinity interactions with cellular receptors including α -dystroglycan, integrins, Lutheran blood group antigen, and sulfated glycolipids.¹⁸ These interactions are critical for basement membrane assembly and provide essential niche cues for numerous stem cell populations including hematopoietic, neural, and mesenchymal stem cells.

Recombinant laminin fragments, particularly the LG1-3 domains of laminin $\alpha 2$ and $\alpha 5$, have emerged as powerful tools for maintaining MSC stemness under defined culture conditions. Recombinant human laminin $\alpha 2$ and $\alpha 5$ LG1-3 domains have been shown to preserve the surface marker profile of MSCs (CD73⁺, CD90⁺, CD105⁺, CD34[−], CD45[−]) over extended culture periods, while suppressing spontaneous differentiation and maintaining trilineage potential.^{19,20} More recently, a novel fusion protein combining FNIII9-10 with an ELP tag (FN9-10ELP) has

demonstrated remarkable efficacy in protecting human MSCs from etoposide-induced premature senescence, a critical consideration for the *ex vivo* expansion of therapeutic cell products.²¹

2.3. Collagen and type I collagen-based platforms

Collagens represent the most abundant structural proteins in the ECM, with type I collagen forming the predominant fibrillar network in connective tissues, bone, and many stem cell niches. The characteristic triple-helical structure of collagen, stabilized by the repetitive Gly-X-Y amino acid sequence (where X and Y are frequently proline and hydroxyproline), confers exceptional mechanical strength and scaffolding capacity. Collagen gels have long served as three-dimensional culture platforms for MSCs, but their undefined composition and variable gelation kinetics have motivated the development of recombinant alternatives.

Hybridization of collagen hydrogels with recombinant bioactive proteins has emerged as a powerful strategy to confer defined biochemical functionality while retaining the superior mechanical and architectural properties of native collagen. Collagen three-dimensional hydrogel matrices loaded with recombinant FGF-2 have demonstrated enhanced MSC proliferation and commitment to the osteogenic lineage compared to unmodified controls, with the collagen network providing sustained FGF-2 retention through electrostatic interactions.²² Bifunctional recombinant proteins that simultaneously bind to collagen and present integrin-binding RGD sequences have been designed to tether bioactive signals to mineralized collagen scaffolds, enabling the creation of osteoinductive microenvironments with clinically relevant properties.²³

2.4. Elastin and elastin-like polypeptides

Elastin is a crosslinked polymer of tropoelastin monomers that confers mechanical resilience to tissues requiring reversible deformation, including blood vessels, skin, and lung parenchyma. Beyond its mechanical role, elastin contains cell-binding sequences—most notably the GRKRR sequence in exon 30—that engage the elastin-binding protein (EBP) receptor complex and activate downstream signaling pathways relevant to vascular cell biology and stem cell mechanosensing.²⁴ The repetitive hydrophobic domains (VPGXG) of elastin exhibit inverse temperature-dependent solubility, forming the conceptual basis for the engineering of elastin-like polypeptides (ELPs) as responsive biomaterial building blocks.

Elastin-like polypeptides (ELPs) are genetically encodable polypeptides derived from the hydrophobic domain of human tropoelastin, characterized by the pentapeptide repeat Val-Pro-Gly-Xaa-Gly (VPGXG),

where the guest residue Xaa determines the lower critical solution temperature (LCST).²⁵ Below the LCST, ELPs are soluble and disordered; above the LCST, they undergo a sharp, reversible phase transition to form coacervates or crosslinked hydrogels, a property exploited for triggered assembly, purification by inverse transition cycling (ITC), and thermoresponsive drug release. ELP fusion proteins combining ECM bioactive domains with ELP tags enable straightforward purification without chromatography and can be designed to self-assemble into nanostructured materials under physiological conditions.²⁶ The chimeric fibrillin-elastin fragment has been evaluated as a substrate for maintaining MSC stemness, where the elastin component provides mechanical compliance while the fibrillin domains regulate transforming growth factor beta (TGF- β) bioavailability in the pericellular space.²⁷

2.5. Fibrillin and the regulation of growth factor bioavailability

Fibrillins are large cysteine-rich glycoproteins (fibrillin-1 and fibrillin-2) that form the backbone of microfibrils in elastic and non-elastic connective tissues. A defining functional characteristic of fibrillins is their capacity to bind latent TGF- β complexes (through latent TGF- β binding proteins, LTBP) and bone morphogenetic protein (BMP) antagonists, thereby sequestering these growth factors within the ECM and regulating their bioavailability in a mechanically responsive manner.²⁸ This growth factor reservoir function makes fibrillins essential architects of stem cell niche homeostasis, particularly in bone marrow, aortic, and ocular niches where TGF- β and BMP signaling balance quiescence and activation.

Recombinant expression of fibrillin domains has been technically challenging due to the disulfide-bond-rich structure requiring oxidative folding environments, but significant progress has been made using secretion-competent mammalian expression systems and insect cell platforms. Chimeric constructs incorporating fibrillin calcium-EGF domains fused to elastin repeats have been expressed in *E. coli* with inclusion body refolding strategies and demonstrated biological activity in MSC niche reconstitution assays, validating the feasibility of producing multidomain ECM-mimetic proteins in scalable bacterial systems.²⁷

2.6. Comparative assessment of recombinant ECM proteins

Although fibronectin, laminin, collagen, elastin, and fibrillin are frequently grouped together as ECM-mimetic building blocks, they differ substantially in their dominant biological function, structural complexity, ease of recombinant production, and consequent suitability

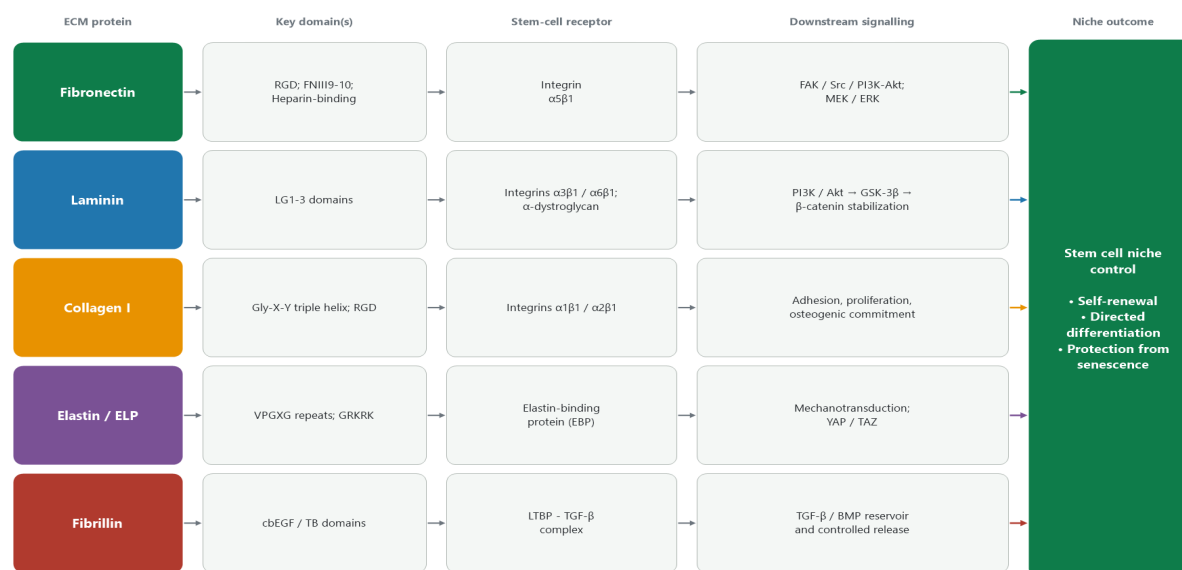


Figure 1. Schematic overview of the five key ECM proteins (fibronectin, laminin, collagen, elastin, and fibrillin), their principal functional domains, the stem cell receptors they engage (integrins, α -dystroglycan, and the elastin-binding protein), and the downstream signalling pathways (FAK/Src/PI3K-Akt, MEK/ERK, YAP/TAZ, and TGF- β /BMP) through which they govern self-renewal, differentiation, and protection from senescence within the stem cell niche.

Abbreviations: ECM: Extracellular matrix; ELP: Elastin-like polypeptide; ERK: Extracellular signal-regulated kinase; LTBP: Latent TGF- β -binding protein; MEK/ERK: Mitogen-activated protein kinase kinase; PI3K: Phosphoinositide 3-kinase; TAZ: Transcriptional coactivator with PDZ-binding motif; TGF- β : Transforming growth factor beta; YAP: Yes-associated protein.

for specific stem cell and tissue engineering applications (Table 2). Fibronectin-derived modules, principally FNIII9-10, are the most versatile and technically accessible: their compact, disulfide-free β -sandwich fold is expressed efficiently in *E. coli*, and their integrin $\alpha 5 \beta 1$ specificity makes them the default choice for generic cell adhesion, osteogenic priming, and anti-senescence coatings.^{13,17,21} Laminin fragments, by contrast, deliver tissue-restricted niche signals—particularly for pluripotent, neural, and hematopoietic populations—but their heterotrimeric architecture and partial dependence on mammalian glycosylation for full receptor engagement raise production cost and complexity.¹⁸⁻²⁰

Collagen offers unrivalled mechanical scaffolding and clinical familiarity, yet the requirement for prolyl hydroxylation to stabilize the triple helix complicates fully recombinant production; collagen platforms are therefore most often deployed as hybrid hydrogels functionalized with recombinant bioactive modules rather than as fully synthetic collagens.^{22,23} Elastin-derived ELPs occupy a distinct role: they contribute tunable, stimulus-responsive mechanics and non-chromatographic purification rather than intrinsic receptor specificity, and are best used as modular carriers into which adhesive or growth-factor-

binding motifs are fused.^{25,26,29} Fibrillin is the most specialized and the most challenging to produce, owing to its cysteine-rich, calcium-dependent domains; its principal value lies in regulating TGF- β and BMP bioavailability, making it uniquely suited to niches in which growth-factor sequestration governs quiescence, such as the perivascular and skeletal stem cell compartments.^{27,28} These complementary strengths and limitations increasingly favour chimeric designs that couple the manufacturability of fibronectin or ELP scaffolds with the specialized signaling functions of laminin or fibrillin, as discussed in section 3.

3. Protein engineering strategies for ECM-mimetic proteins

3.1. Recombinant expression systems

The choice of expression host profoundly influences the yield, folding, post-translational modification profile, and functional integrity of recombinant ECM proteins. Each system offers distinct advantages and limitations that must be considered in the context of the specific protein target and intended application.

E. coli remains the most widely used expression platform

due to its rapid growth, low cost, genetic tractability, and scalability. It is particularly well-suited for the production of structurally simple ECM fragments and ELP fusions that do not require complex glycosylation. Strategies including T7 promoter-based overexpression, co-expression of molecular chaperones, refolding from inclusion bodies, and periplasmic secretion have all been employed to maximize yields of functionally active ECM fragments.³⁰ The ITC purification method—exploiting the phase transition of ELP tags—enables non-chromatographic purification that is readily scalable and avoids the immunogenicity concerns associated with metal-affinity tags retained in the final product.

For ECM proteins requiring mammalian-type glycosylation or complex disulfide bond formation, Chinese Hamster Ovary (CHO) cells, Human Embryonic Kidney (HEK293) cells, and insect cell lines (Sf9 with baculovirus) provide superior folding environments at the cost of higher production expenses.³¹ Yeast systems such as *Pichia pastoris* offer a compromise, providing secretion-competent expression with some glycosylation capacity and high volumetric yields. For the most complex structural ECM proteins such as full-length fibronectin, collagen, and fibrillin, cell-free translation systems and transient transfection in HEK293 cells have enabled production of research quantities sufficient for biological characterization.

3.2. Domain engineering and functional fragment design

A foundational principle in ECM-mimetic protein design is the identification and isolation of minimal functional domains that recapitulate the biological activity of the parent molecule. This reductionist approach, while sacrificing some biological complexity, enables the production of structurally defined proteins with predictable activity and facilitates mechanistic studies of specific receptor-ligand interactions. The FNIII9-10 module, comprising approximately 200 amino acids, has been the paradigmatic example: this minimal fragment retains full integrin $\alpha 5 \beta 1$ binding capacity and supports cell adhesion, spreading, and mechanosensing with affinities comparable to or exceeding those of intact fibronectin adsorbed to surfaces.^{13,17}

Rational mutagenesis of functional domains has enabled the engineering of variants with enhanced binding affinity, altered receptor selectivity, or resistance to proteolytic degradation. Phage display and directed evolution approaches have yielded fibronectin and laminin variants with sub-nanomolar affinities for specific integrin heterodimers, enabling the construction of highly selective

niche environments that preferentially expand specific stem cell populations.³² Introduction of non-natural amino acids at defined positions through amber suppression technology provides orthogonal reactive handles for site-specific conjugation of small molecules, imaging agents, or biomaterial matrices, preserving the bioactive orientation of the protein on surfaces and preventing receptor blocking by random amine coupling.³³

3.3. Elastin-like polypeptide fusion strategy

The fusion of bioactive ECM domains to ELP tags represents one of the most elegant and practically impactful protein engineering strategies developed in the past two decades.²⁵ The modular genetic architecture of ELP fusions—[ECM domain]-[ELP tag] or [ELP tag]-[ECM domain]-[ELP tag]—enables straightforward customization of the LCST through variation of the guest residue X in VPGXG, the number of pentapeptide repeats (typically 40–180 units), and the flanking sequences. At temperatures below the LCST, ELP fusions are soluble monomers; above the LCST, they undergo phase separation, which can be engineered to drive self-assembly into nanoparticles, worm-like micelles, or physically crosslinked hydrogels depending on the architecture.²⁹

Beyond purification, the modular genetic architecture of ELP fusions has been widely exploited to create defined, cell-instructive substrates. Pioneering work from the Tirrell and Heilshorn laboratories demonstrated that artificial ECM proteins combining an elastin-derived backbone with fibronectin-derived RGD and CS5 cell-binding domains support robust cell adhesion and proliferation while permitting the adhesion-ligand density and the matrix mechanics to be tuned independently of one another.³⁴ Chemically crosslinked ELP hydrogels developed by Chilkoti and co-workers³⁵ further showed that the swelling ratio and elastic modulus of ELP networks can be programmed through ELP chain length and crosslinker density, yielding matrices whose stiffness can be matched to a target stem cell niche. Hybrid ELP-poly(ethylene glycol) networks have extended this approach by decoupling matrix stiffness from cell-ligand concentration in optically transparent, cytocompatible gels suitable for three-dimensional culture.³⁶ Collectively, these studies illustrate how ELP fusion technology converts ECM-derived bioactive motifs into scalable, stimulus-responsive materials whose biochemical and biophysical properties can be specified at the genetic level.^{26,29}

3.4. Chimeric multi-domain and chimeric growth factor receptor proteins

Chimeric multi-domain proteins represent the most sophisticated tier of ECM-mimetic protein engineering,

integrating two or more functional modules from distinct proteins into a single polypeptide chain to achieve synergistic biological activity not achievable by individual components.³⁷ The design logic typically involves: (i) identification of complementary receptor-binding domains; (ii) selection of appropriate flexible linker sequences (commonly (GGGS)_n repeats) to preserve domain folding and minimize steric interference; (iii) optimization of domain order based on structural constraints; and (iv) validation of multi-receptor engagement by orthogonal binding assays.

A particularly impactful example is the design of chimeric FGF21/HGF receptor (HGFR) binding proteins for the treatment of non-alcoholic fatty liver disease (NAFLD). FGF21, a hepatokine that improves insulin sensitivity and lipid metabolism, and HGF, the ligand for the c-Met receptor that drives hepatocyte regeneration, were fused into a single chimeric molecule that simultaneously engages FGFR1c/ β -KL and c-Met receptors on hepatocytes.³⁸ This bifunctional protein demonstrated superior efficacy compared to either component alone in reversing lipid accumulation in NAFLD hepatocyte models, illustrating how chimeric growth-factor fusion strategies can be leveraged to address complex multifactorial diseases. A complementary design philosophy has been advanced by Hubbell and colleagues, who engineered multifunctional fibronectin fragments in which the integrin-binding FNIII9-10 module is genetically linked to the growth-factor-binding FNIII12-14 domain; when incorporated into fibrin matrices, these constructs concurrently present integrin ligands and sequester morphogens such as BMP-2, PDGF-BB, and VEGF, markedly enhancing wound and bone healing at low growth-factor doses.³⁹ The same group subsequently generated growth factors engineered for super-affinity to the ECM by appending a promiscuous heparin-binding domain, which dramatically prolonged matrix retention and therapeutic efficacy *in vivo*.⁴⁰ Together, these chimeric architectures demonstrate how a single engineered polypeptide can recapitulate the combinatorial integrin- and growth-factor-signaling functions normally distributed across multiple native matrix components.

3.5. Modular assembly and computational protein design

The engineering strategies described above can be arranged along a spectrum of increasing design freedom, each with characteristic advantages and limitations (Figure 2; Table 3). Domain truncation is the simplest and most robust approach, yielding defined, readily expressed fragments at the cost of the cooperative interactions present in the full-length parent protein. Fusion-protein strategies—

exemplified by ELP tagging—add responsive mechanics and purification handles but can compromise long-term stability *in vivo*. Modular assembly extends this logic by concatenating several orthogonal binding modules through structured or elastomeric linkers to reconstruct combinatorial signaling within a single chain, at the expense of more demanding cloning and a higher risk of misfolding.^{37,39,40}

Computational and machine-learning-guided protein design now constitutes a fourth, rapidly maturing tier. Deep-learning structure prediction—AlphaFold2 and the subsequent AlphaFold3 model, which extends accurate prediction to protein–ligand and protein–nucleic acid complexes—enables *in silico* validation of engineered domain geometries and interface compatibility before experimental production.^{41,42} Generative approaches such as RFdiffusion permit the *de novo* design of proteins with bespoke integrin-binding pockets, tunable stability, and engineered protease-labile sites for programmable degradation, moving the field beyond the recombination of natural domains toward fully synthetic ECM-mimetic scaffolds.⁴³ These tools substantially shorten design–build–test cycles, but they remain limited by the scarcity of high-quality functional (as opposed to purely structural) training data, imperfect prediction of dynamic and post-translationally modified states, and the continuing need for experimental validation of biological activity. The practical trajectory of the field is therefore a convergence in which computational design proposes candidate architectures that are realized through modular assembly and fusion strategies and manufactured in the expression systems described in section 3.1.

4. Applications in stem cell niche engineering

4.1. Maintaining stemness and self-renewal

The *ex vivo* expansion of therapeutically relevant stem cell populations—including MSCs, hematopoietic stem cells (HSCs), and neural stem cells (NSCs)—faces the fundamental challenge that replicative expansion in conventional tissue culture plastic induces rapid loss of multipotency, upregulation of stress-response genes, and ultimately replicative senescence. ECM-mimetic recombinant proteins offer a chemically defined strategy to recapitulate the *in vivo* niche signals that enforce stemness while permitting the population expansion required for clinical doses^{44–46}

Recombinant human laminin α 2 LG1-3 and laminin α 5 LG1-3 domains have been evaluated as defined substrates for MSC culture across multiple passages. Human MSCs cultured on these substrates demonstrated sustained

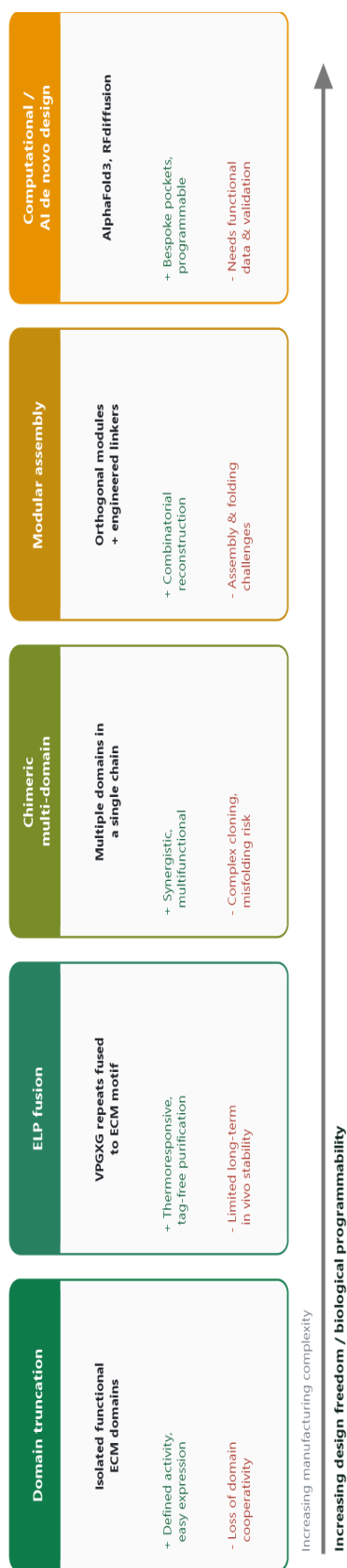


Figure 2. Spectrum of protein engineering strategies for ECM-mimetic proteins, progressing from domain truncation and ELP fusion through chimeric multi-domain and modular assembly to computational and AI-guided *de novo* design, illustrating the increasing design freedom and the corresponding trade-offs in manufacturability and biological complexity. Abbreviations: AI: Artificial intelligence; ECM: Extracellular matrix; ELP: Elastin-like polypeptide.

expression of stemness markers (CD73, CD90, CD105) and retention of trilineage differentiation potential through passage 10, whereas cells on uncoated tissue culture plastic showed progressive loss of CD105 expression and reduced chondrogenic capacity by passage 6.^{19,20} Mechanistically, laminin LG domain engagement of α -dystroglycan and integrins $\alpha 3 \beta 1 / \alpha 6 \beta 1$ activates the PI3K/Akt pathway, which phosphorylates and inactivates GSK-3 β , thereby stabilizing β -catenin and maintaining a transcriptional program compatible with multipotency.⁴⁷

Self-assembled fibronectin matrix protein constructs incorporating oligomerization domains have been engineered to mimic the nanofibrillar architecture of native FN matrices assembled by cells. These engineered matrices, which form fibrils of defined diameter through head-to-tail oligomerization, demonstrated enhanced activation of focal adhesion kinase (FAK) and downstream ERK1/2 phosphorylation compared to monomeric FNIII9-10 substrates, translating into superior maintenance of MSC clonogenicity over extended culture.⁴⁸

4.2. Directed differentiation

Directed differentiation of stem cells through ECM-mimetic substrates exploits the mechanochemical coupling between integrin engagement, cytoskeletal tension, and nuclear mechanotransduction to bias lineage commitment without reliance on soluble morphogens that may be difficult to remove prior to clinical use. The design of differentiation-instructive ECM-mimetic proteins requires knowledge of the specific integrin-ECM interactions that activate lineage-instructive downstream pathways.

For osteogenic commitment of MSCs, proteins incorporating the FNIII9-10 domain for integrin $\alpha 5 \beta 1$ engagement, combined with heparin-binding domains for BMP-2 or FGF-18 sequestration, have demonstrated robust osteoinductive activity. A recombinant fibronectin type III domain fused to an ELP tag (FNIII-ELP) coated on poly(caprolactone) (PCL) nanofibers significantly enhanced alkaline phosphatase activity and calcium deposition by MSCs under basal (osteogenic supplement-free) conditions, suggesting that substrate-mediated mechanosensing alone was sufficient to drive osteogenic commitment.⁴⁹ The addition of FGF-18 sequestered to the heparin-binding domain of the same construct further amplified osteogenesis through FGFR3-mediated activation of the BMP-SMAD pathway.

Cyclic mechanical stretch has been identified as a potent co-stimulator of cellular reprogramming when combined with ECM-mimetic substrates. Cyclic stretch promotes cytoskeletal-nuclear mechano-coupling through the LINC (linker of nucleoskeleton and cytoskeleton) complex,

Table 3. Protein engineering strategies for ECM-mimetic recombinant proteins: comparative analysis

Strategy	Key features	Advantages	Limitations
Domain truncation	Isolated functional ECM domains	Defined activity, easier expression	Loss of cooperative domain effects
ELP fusion	VPGXG repeat fused to ECM domain	Thermo-responsive, self-assembling, LCST tunable	Limited long-term stability <i>in vivo</i>
Chimeric multi-domain	Multiple functional domains in single chain	Synergistic signaling, multifunctionality	Complex cloning, potential misfolding
Heparin-binding peptide	Heparin-binding domain for growth factor sequestration	Sustained growth factor retention	Dependent on heparin availability
Site-specific conjugation	Unnatural amino acid or click chemistry	Precise orientation control	Requires specialized expression

Abbreviations: ECM: Extracellular matrix; ELP: Elastin-like polypeptide; LCST: Lower critical solution temperature.

driving epigenetic remodeling of pluripotency loci through the upregulation of histone demethylase KDM5B and concurrent activation of YAP/TAZ mechanosensory transcription factors⁵⁰⁻⁵² ECM-mimetic substrates with tunable stiffness—achieved through the crosslinking density of ELP hydrogels—have emerged as enabling tools for dissecting the independent contributions of stiffness, adhesion ligand density, and cyclic strain in directing stem cell lineage commitment.

4.3. Protection from cellular senescence

Cellular senescence—defined as a permanent cell cycle arrest accompanied by a pro-inflammatory senescence-associated secretory phenotype (SASP)—is a major obstacle to the clinical translation of cell therapies. *Ex vivo* expansion under conventional culture conditions inevitably induces replicative senescence, while the oxidative and genotoxic stresses encountered during manufacturing compound this challenge. Senescent MSCs exhibit impaired immunomodulatory function, reduced secretion of trophic factors, and enhanced pro-inflammatory cytokine production, collectively rendering them less suitable as therapeutic agents.⁵³

FN9-10ELP has been reported to protect MSCs from etoposide-induced premature senescence, an effect consistent with engagement of integrin $\alpha 5 \beta 1$ by the FNIII9-10 module and downstream PI3K/Akt signaling, which has been associated with destabilization of p21Cip1, a critical executor of the senescent growth arrest.²¹ The protective effect was accompanied by reduced expression of selected SASP markers (IL-6, IL-8, PAI-1) and partial preservation of the MSC immunosuppressive transcriptome. These findings establish ECM-mimetic protein coatings as a viable strategy for maintaining the functional potency of therapeutic MSC products during manufacturing.

5. Biomaterial platforms incorporating ECM-mimetic recombinant proteins

5.1. Electrospun nanofiber scaffolds

Electrospinning generates nonwoven fibrous meshes with fiber diameters ranging from tens of nanometers to several micrometers, mimicking the physical architecture of native ECM fibrillar networks. The topographic guidance cues provided by aligned fibers, combined with the high surface-area-to-volume ratio that facilitates efficient protein display, make electrospun scaffolds ideal platforms for the presentation of ECM-mimetic recombinant proteins. PCL, poly(lactic-co-glycolic acid) (PLGA), and collagen-PCL composite fibers have all been used as fiber substrates for recombinant protein functionalization.⁵⁴

Covalent coupling strategies—employing EDC/NHS chemistry, plasma activation, or maleimide-thiol click reactions—are preferred over simple physisorption for long-term retention of protein functionality under cell culture conditions and mechanical loading. FN-peptide-amphiphile coupling to PCL fibers through carbodiimide chemistry significantly accelerated initial cellular attachment, spreading, and cytoskeletal organization compared to unmodified fibers, with the peptide architecture preserving integrin-binding conformation that is frequently compromised during adsorption of full-length proteins.⁵⁵ Graphene oxide-functionalized nanofiber composite matrices incorporating ECM-mimetic protein coatings have been shown to synergistically enhance the differentiation of hippocampal neuronal cells, with the graphene oxide providing electrical conductivity that augments the downstream signaling initiated by integrin-ECM engagement.⁵⁶

5.2. Hydrogels and injectable scaffolds

Elastin-like polypeptide (ELP)-based hydrogels have emerged as paradigmatic examples of protein-

engineered materials that combine precise biochemical programmability with tunable mechanical properties, minimal immunogenicity (arising from their human tropoelastin sequence origin), and the ability to undergo sol-gel transitions under physiologically relevant conditions. Thermally crosslinked ELP hydrogels incorporating RGDS and heparin-binding domains can be designed to gel at 37°C, enabling minimally invasive injection and in situ scaffold formation at the target tissue site.⁵⁷

Hybrid protein-polysaccharide hydrogels that combine the bioactivity of recombinant ECM proteins with the structural and rheological properties of natural polysaccharides such as hyaluronic acid, chitosan, or alginate represent a versatile platform for three-dimensional stem cell encapsulation. Gelatin-siloxane hybrid scaffolds functionalized with recombinant SDF-1 (stromal cell-derived factor-1/CXCL12) demonstrated potent chemoattractant activity for MSCs and endothelial progenitor cells *in vitro* and *in vivo*, establishing a framework for scaffold-guided stem cell recruitment to injury sites without *ex vivo* cell expansion.⁵⁸ Recombinant SDF-1 co-expressed with neurotrophic elements has further been shown to promote neurite outgrowth in PC-12 cells, suggesting that cytokine-functionalized hydrogels can function as neural regeneration matrices.⁵⁹

5.3. Implant surface functionalization

Titanium and its alloys are the predominant materials for dental and orthopedic implants owing to their excellent biocompatibility and osseointegration capacity. However, the bioinert titanium oxide surface provides minimal instructive cues for bone-forming cell attachment, and peri-implant infection remains a leading cause of implant failure. ECM-mimetic recombinant protein coatings address both challenges: bioactive proteins promote osseointegration while antimicrobial modifications reduce infection risk.

FGF-fibronectin fusion proteins covalently coupled to anodized titanium surfaces via silane coupling agents demonstrated significantly enhanced osseointegration in preclinical models, with the fibronectin domain driving early osteoblast attachment and the FGF component promoting vascularization of the peri-implant space.⁶⁰ Truncated vascular endothelial cadherin (VE-cadherin) extracellular 1-4 domain proteins immobilized on small-diameter synthetic vascular grafts promoted rapid endothelialization and maintained endothelial monolayer integrity under arterial shear stress conditions, addressing the critical challenge of thrombogenicity in small-diameter vascular prostheses.⁶¹ More recently, multifunctional gallium-defensin surface coatings combining the

antimicrobial activity of gallium ions with the membrane-disrupting activity of recombinant defensin peptides demonstrated dual antibacterial and anti-inflammatory activity on implantable device surfaces, without compromising the viability of adhering host cells.⁶²

5.4. Nanoparticle-based delivery systems

Protein corona formation on nanoparticle surfaces—the adsorption of serum proteins that occurs immediately upon exposure to biological fluids—critically determines the biodistribution, cellular uptake, and cytotoxicity of nanoparticles *in vivo*. Understanding and engineering the protein corona has emerged as a key strategy for controlling the biological fate of therapeutic nanoparticles. Studies using nanodiamonds have demonstrated that the composition and conformation of the adsorbed protein corona, particularly fibronectin and vitronectin, determines integrin-mediated cellular uptake pathways and subsequent intracellular trafficking.⁶³

Mesoporous silica nanoparticles (MSNs) functionalized with heparin-binding ECM proteins have been developed as growth factor delivery vehicles that leverage the high-affinity electrostatic sequestration of heparin-binding proteins to achieve sustained, controlled release kinetics. MSNs loaded with BMP-2 plasmid DNA and coated with FN heparin-binding domain fragments demonstrated significantly elevated transfection efficiency and sustained osteogenic gene expression in MSCs compared to conventional lipofection, attributed to the integrin-mediated endocytic uptake pathway facilitated by the FN coating.⁶⁴ Bioactive inorganic core/chitosan shell nanohybrids self-assembled from calcium silicate nanoparticles and chitosan incorporating ECM-mimetic proteins demonstrated tissue regenerative activity through the co-delivery of bioactive silicon ions and ECM adhesion signals, establishing a paradigm for multi-modal nanocarrier design.⁶⁵

5.5. Emerging applications in advanced biofabrication

Beyond the established platforms described above, ECM-mimetic recombinant proteins are increasingly central to a new generation of biofabrication technologies that demand defined, printable, and biologically instructive materials. In three-dimensional (3D) bioprinting, recombinant ECM proteins and ELP-based bioinks provide the tunable viscosity, shear-thinning behavior, and cell-instructive ligand presentation required to deposit living constructs with spatial precision; genetically encoded control over crosslinking and mechanics allows the printed matrix to be matched to the target tissue while retaining a batch-defined composition.^{66,67} Such defined bioinks directly

address a key limitation of animal-derived materials such as Matrigel, whose lot-to-lot variability confounds the reproducibility of printed constructs.

Organoids and organ-on-chip systems present a complementary opportunity. The reproducibility and clinical translatability of organoid models are presently constrained by their reliance on undefined tumour-derived basement-membrane extracts; chemically defined recombinant ECM-mimetic hydrogels, in which adhesion-ligand identity and matrix mechanics are specified independently, are emerging as synthetic replacements that improve batch consistency and enable mechanistic dissection of niche cues.⁶⁶ In microfluidic organ-on-chip devices, recombinant ECM coatings and membranes recreate tissue-specific basement membranes and support the physiologically relevant cell–matrix interactions required for disease modeling, drug screening, and personalized-medicine applications.⁶⁸ Looking forward, the integration of patient-derived cells with defined, computationally designed ECM-mimetic matrices points toward personalized regenerative medicine, in which stem cell niches are tailored to an individual's tissue mechanics and signalling requirements, and toward closed-loop biofabrication workflows that couple *in silico* design with automated, Good Manufacturing Practice (GMP)–compatible production (Figure 3).

6. Clinical translation: Challenges and perspectives

6.1. Scalability and manufacturing

The transition from research-scale to clinical-grade manufacturing of recombinant ECM proteins presents substantial challenges related to yield, purity, cost of goods, and process reproducibility. For bacterial expression systems, fed-batch fermentation in stirred-tank bioreactors can achieve volumetric productivities of 1–10 g/L for structurally simple ECM fragments and ELP fusions, providing an economically viable route to clinical-scale quantities.⁶⁹ The ITC purification strategy, which requires only thermal cycling and centrifugation, is particularly attractive for GMP manufacturing as it eliminates the column chromatography steps that are major cost drivers in biopharmaceutical production.

For more complex ECM proteins requiring mammalian expression, the cost of goods is substantially higher. Optimization of transient expression in CHO or HEK293 cells using high-density perfusion bioreactor systems and serum-free, chemically defined media formulations has reduced production costs significantly, but regulatory compliance with GMP standards remains technically demanding. Process analytical technology (PAT) approaches incorporating inline spectroscopic monitoring of protein folding state and aggregation provide real-time quality assurance during manufacturing, a critical requirement for consistency of biological activity across production batches.

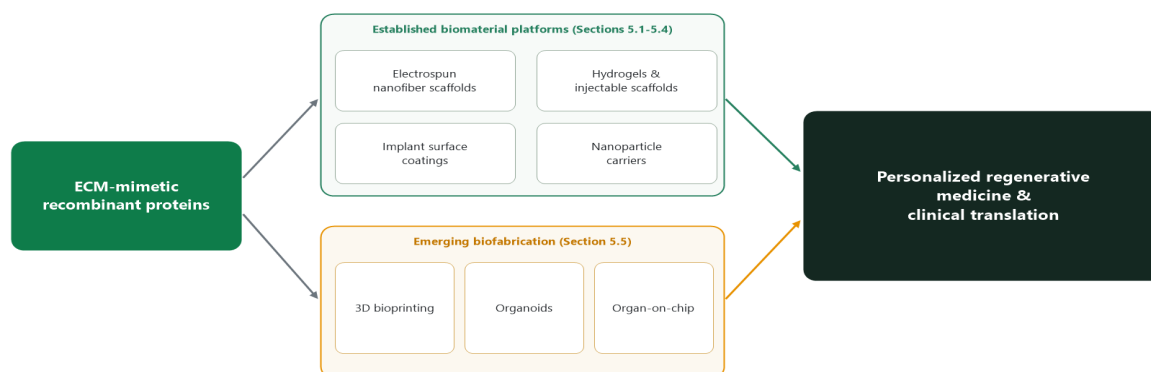


Figure 3. Integration of ECM-mimetic recombinant proteins into biomaterial platforms (electrospun scaffolds, injectable hydrogels, implant surface coatings, and nanoparticle carriers) and into emerging biofabrication technologies (3D bioprinting, organoids, and organ-on-chip systems), and the resulting translational pipeline toward personalized regenerative medicine.

Abbreviation: ECM: Extracellular matrix.

Batch-to-batch consistency is the single most important manufacturing attribute distinguishing recombinant ECM proteins from the animal-derived matrices they are intended to replace, and it is a principal driver of their regulatory acceptability. Because recombinant proteins are produced from a defined master and working cell bank against a fixed genetic sequence, they can, in principle, achieve far tighter lot-to-lot reproducibility than Matrigel or decellularized ECM. Realizing this advantage under GMP requires a fully qualified cell bank, a locked-down and validated upstream and downstream process, and a comprehensive panel of release specifications—identity by mass spectrometry and peptide mapping, purity and aggregate content by size-exclusion chromatography, endotoxin and bioburden limits, residual host-cell protein and DNA, and a quantitative potency assay tied to the intended biological function. Design-of-experiments-based process characterization and quality-by-design principles, combined with the process analytical technology approaches noted above, provide the framework for demonstrating the consistency that regulators require of clinical-grade material.

6.2. Immunogenicity assessment

The immunogenicity of recombinant ECM proteins is a critical safety consideration that must be thoroughly characterized prior to clinical application. Although proteins derived from human ECM sequences have inherently low immunogenic potential compared to xenogeneic or non-self sequences, several factors can induce anti-drug antibody responses: post-translational modifications differing from the endogenous protein (e.g., non-human glycosylation from bacterial or yeast expression), protein aggregates that present multivalent epitopes, oxidized methionine or deamidated asparagine residues generated during storage, and leachable manufacturing impurities.⁷⁰

ELP-based proteins derived from the human tropoelastin sequence have demonstrated minimal immunogenicity in repeated-dose rodent and non-human primate studies, with no detectable IgG or IgM anti-drug antibody responses observed at therapeutic doses. For chimeric proteins combining sequences from multiple human proteins, *in silico* T-cell epitope prediction tools followed by *in vitro* peripheral blood mononuclear cell (PBMC) proliferation assays provide a stratified risk assessment framework aligned with ICH S8 guidelines. De-immunization by substitution of predicted T-cell epitope residues with functionally conservative alternatives has been successfully applied to FNIII domains and growth factor fusions to reduce immunogenic risk while preserving receptor-binding activity.⁷¹

A tiered immunogenicity risk assessment is now expected by regulators for novel recombinant biomaterials. *In silico* prediction of major histocompatibility complex class II-binding T-cell epitopes provides an initial screen, followed by *in vitro* confirmation using MHC-associated peptide proteomics (MAPPs) and PBMC proliferation assays, and ultimately by *in vivo* evaluation of anti-drug antibody formation in appropriate animal models. For products intended for implantation or repeated dosing, this assessment should also encompass the innate immune and foreign-body responses to the assembled biomaterial, because the aggregation state, particulate load, and matrix stiffness can modulate macrophage polarization independently of adaptive immunogenicity. Aligning these analyses with ICH and regional regulatory guidance early in development substantially de-risks later clinical translation.

6.3. Regulatory pathway considerations

The regulatory classification of recombinant ECM proteins depends critically on their intended use and the nature of the product. When used as culture substrates for the *ex vivo* expansion of stem cells intended for autologous or allogeneic transplantation, recombinant ECM proteins are regulated as components of the cell therapy manufacturing process and must meet USP/EP standards for bioprocess reagents, including sterility, mycoplasma, viral clearance, and residual host cell protein/DNA limits. When incorporated into implantable biomaterial scaffolds, the combined product is classified as a combination product under FDA 21 CFR Part 3, requiring a marketing authorization that addresses both the device and biological components.

The European Medicines Agency (EMA) framework for advanced therapy medicinal products (ATMPs) provides a well-defined pathway for recombinant protein-functionalized tissue-engineered products under the ATMP regulation (EC 1394/2007). The ATMP classification requires full characterization of the recombinant protein component, including mass spectrometry-based sequence confirmation, glycan analysis, and potency assays that demonstrate biological activity relevant to the claimed clinical indication. The development of potency assay toolkits—including standardized cell adhesion, stemness maintenance, and differentiation assays—is a pressing need for the field that would facilitate regulatory review and enable head-to-head comparison across manufacturing sites.

6.4. Current clinical status and future directions

As of 2025, no recombinant ECM-mimetic protein has achieved regulatory approval as a standalone therapeutic,

but several are in advanced preclinical or early clinical development. Recombinant fibronectin fragments are used clinically in the manufacturing of T-cell and NK-cell products (RetroNectin, Takara Bio), where their fibronectin CH-296 fragment facilitates co-localization of T cells with retroviral vectors to enhance transduction efficiency—an established clinical application that validates the safety and GMP-compatibility of recombinant FN fragments.⁷² Recombinant laminin-521 (BioLamina) is used commercially for the culture of human pluripotent stem cells under xeno-free conditions and represents an important proof-of-concept for defined recombinant laminin products in clinical-grade cell manufacturing.

The current translational and clinical development status of representative ECM-mimetic recombinant proteins and platforms is summarized in Table 4. At present the landscape is dominated by defined recombinant reagents used within cell-manufacturing workflows—such as the fibronectin CH-296 fragment and recombinant laminin isoforms—rather than by ECM-mimetic proteins

approved as standalone therapeutics, underscoring both the maturity of the reagent role and the translational gap that remains for *in vivo* therapeutic products.

The convergence of computational protein design, machine learning-guided directed evolution, and advanced manufacturing technologies is poised to dramatically accelerate the development of the next generation of ECM-mimetic proteins. AlphaFold2-guided *de novo* design of ECM-mimetic proteins with engineered integrin-binding pockets, protease cleavage sites for on-demand degradation, and cryptic bioactive domain exposure is a realistic near-term goal.⁴¹ Cell-free protein synthesis (CFPS) systems offer rapid prototyping capabilities for novel ECM-mimetic protein variants, enabling iterative design-build-test cycles without the delays of microbial fermentation. Three-dimensional bioprinting of complex tissue constructs using ECM-mimetic protein-based bioinks is progressing toward clinical-grade vascular grafts, corneal replacements, and bone substitutes that could enter first-in-human trials within the coming decade.

Table 4. Translational and clinical development status of representative ECM-mimetic recombinant proteins and platforms

Product/Platform	Type	Development status	Representative use/Notes
RetroNectin (fibronectin CH-296)	Recombinant FN fragment	Commercial; clinical cell manufacturing	GMP reagent for retroviral transduction of T/NK-cell products
Recombinant laminin-521	Recombinant laminin isoform	Commercial; research/clinical use	Xeno-free substrate for pluripotent stem cell culture
FNIII-ELP/FN9-10ELP coatings	ELP fusion protein	Preclinical	Osteoinductive and anti-senescence substrates for MSCs
Chimeric FGF21/HGFR	Chimeric growth-factor fusion	Preclinical	Bifunctional therapeutic in NAFLD hepatocyte models
ELP/hybrid recombinant hydrogels and bioinks	Injectable/printable scaffold	Preclinical	3D bioprinting, organoid and organ-on-chip matrices
AI-designed <i>de novo</i> ECM proteins	Computationally designed protein	Early research	Prescribed integrin selectivity and programmable degradation

Abbreviations: AI: Artificial intelligence; ECM: Extracellular matrix; ELP: Elastin-like polypeptide; FGF: Fibroblast growth factor; FN: Fibronectin; GMP: Good Manufacturing Practice; HGFR: Hepatocyte growth factor receptor; MSC: Mesenchymal stem cell; NAFLD: Nonalcoholic fatty liver disease.

7. Conclusions

This review has comprehensively examined the molecular biology of key ECM components, the protein engineering strategies available for their recombinant production and functional enhancement, and the diverse biomaterial applications that are moving ECM-mimetic proteins toward clinical translation. Several key conclusions emerge from this analysis.

First, the rational design of ECM-mimetic recombinant proteins has matured from the production of simple functional fragments to the engineering of sophisticated

multi-domain chimeric proteins with synergistic biological activities. ELP fusion technology has been transformative in enabling scalable purification and self-assembling material formation, while chimeric protein design has unlocked multi-receptor co-engagement strategies that more faithfully replicate the combinatorial complexity of native ECM signaling. Second, the breadth of biomaterial platforms amenable to ECM-mimetic protein functionalization—spanning electrospun nanofibers, injectable hydrogels, implant surfaces, and nanoparticle delivery systems—attests to the versatility of these proteins as enabling components for next-generation regenerative medicine products.

Third, the clinical translation of ECM-mimetic proteins faces solvable but non-trivial challenges in manufacturing scalability, immunogenicity characterization, and regulatory pathway navigation. The field would benefit significantly from the development of harmonized potency assays, industry-wide manufacturing standards, and dedicated regulatory guidance documents that acknowledge the unique characteristics of protein-functionalized biomaterial combination products.

Finally, the integration of machine learning-guided protein design, CRISPR-based expression optimization, and biofabrication technologies is creating an unprecedented opportunity to accelerate both the discovery and manufacturing of ECM-mimetic therapeutics. As these technologies converge, it is anticipated that defined, recombinant ECM-mimetic proteins will transition from laboratory tools to clinical-grade materials that fundamentally transform the quality and reproducibility of stem cell-based therapies and implantable regenerative medicine products.

Looking ahead, four priorities are identified that are likely to most powerfully shape the coming decade of the field. First, AI-assisted protein design—coupling generative models such as RFdiffusion with high-accuracy structure prediction—should progress from proof-of-concept toward the routine *de novo* creation of ECM-mimetic proteins with prescribed integrin selectivity, growth factor affinity, and programmable degradation.^{42,43} Second, programmable ECM biomaterials that sense and respond to cellular or environmental cues—through engineered protease-labile linkers, logic-gated ligand presentation, and stimulus-responsive mechanics—will enable dynamic rather than static control of stem cell fate. Third, the convergence of these defined materials with patient-derived cells will allow the construction of personalized stem cell niches tailored to individual tissue mechanics and signaling requirements. Fourth, clinical impact will require concerted investment in translational infrastructure: harmonized potency assays, GMP-compatible and scalable manufacturing, standardized immunogenicity and regulatory frameworks, and closed-loop pipelines that link computational design directly to automated production. Pursued in an integrated manner, progress across these four fronts will be decisive in transforming ECM-mimetic recombinant proteins from enabling laboratory reagents into broadly deployed clinical products.

Acknowledgments

None.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2026-25589777).

Conflict of interest

The author declares no competing interests.

Author contributions

This is a single-authored article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. *J Cell Sci.* 2010;123(24):4195–4200.
doi: 10.1242/jcs.023820
2. Hynes RO. The Extracellular Matrix: Not Just Pretty Fibrils. *Science.* 2009;326(5957):1216–1219.
doi: 10.1126/science.1176009
3. Watt FM, Huck WTS. Role of the extracellular matrix in regulating stem cell fate. *Nat Rev Mol Cell Biol.* 2013;14(8):467–473.
doi: 10.1038/nrm3620
4. Gattazzo F, Urciuolo A, Bonaldo P. Extracellular matrix: A dynamic microenvironment for stem cell niche. *Biochim Biophys Acta Gen Subj.* 2014;1840(8):2506–2519.
doi: 10.1016/j.bbagen.2014.01.010
5. Lu P, Takai K, Weaver VM, Werb Z. Extracellular Matrix Degradation and Remodeling in Development and Disease. *Cold Spring Harb Perspect Biol.* 2011;3(12):a005058.
doi: 10.1101/cshperspect.a005058
6. Hynes RO, Naba A. Overview of the Matrisome--An Inventory of Extracellular Matrix Constituents and Functions. *Cold Spring Harb Perspect Biol.* 2011;4(1):a004903.
doi: 10.1101/cshperspect.a004903
7. Hughes CS, Postovit LM, Lajoie GA. Matrigel: A complex protein mixture required for optimal growth of cell culture. *Proteomics.* 2010;10(9):1886–1890.

- doi: 10.1002/pmic.200900758
8. Crapo PM, Gilbert TW, Badyalak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials*. 2011;32(12):3233-3243.
doi: 10.1016/j.biomaterials.2011.01.057
 9. Langer R, Tirrell DA. Designing materials for biology and medicine. *Nature*. 2004;428(6982):487-492.
doi: 10.1038/nature02388
 10. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotechnol*. 2005;23(1):47-55.
doi: 10.1038/nbt1055
 11. Straley KS, Heilshorn SC. Independent tuning of multiple biomaterial properties using protein engineering. *Soft Matter*. 2009;5(1):114-124.
doi: 10.1039/b808504h
 12. Pankov R, Yamada KM. Fibronectin at a glance. *J Cell Sci*. 2002;115(20):3861-3863.
doi: 10.1242/jcs.00059
 13. Martino MM, Hubbell JA. The 12th–14th type III repeats of fibronectin function as a highly promiscuous growth factor-binding domain. *FASEB J*. 2010;24(12):4711-4721.
doi: 10.1096/fj.09-151282
 14. Ruoslahti E. RGD and other recognition sequences for integrins. *Annu Rev Cell Dev Biol*. 1996;12:697-715.
doi: 10.1146/annurev.cellbio.12.1.697
 15. Chen XD, Dusevich V, Feng JQ, Manolagas SC, Jilka RL. Extracellular Matrix Made by Bone Marrow Cells Facilitates Expansion of Marrow-Derived Mesenchymal Progenitor Cells and Prevents Their Differentiation Into Osteoblasts. *J Bone Miner Res*. 2007;22(12):1943-1956.
doi: 10.1359/jbmr.070725
 16. Wijelath ES, Rahman S, Namekata M, et al. Heparin-II Domain of Fibronectin Is a Vascular Endothelial Growth Factor-Binding Domain. *Circ Res*. 2006;99(8):853-860.
doi: 10.1161/01.res.0000246849.17887.66
 17. Martino MM, Mochizuki M, Rothenfluh DA, Rempel SA, Hubbell JA, Barker TH. Controlling integrin specificity and stem cell differentiation in 2D and 3D environments through regulation of fibronectin domain stability. *Biomaterials*. 2009;30(6):1089-1097.
doi: 10.1016/j.biomaterials.2008.10.047
 18. Aumailley M. The laminin family. *Cell Adh Migr*. 2013;7(1):48-55.
doi: 10.4161/cam.22826
 19. Lee S, Lee DS, Jang JH. Recombinant laminin $\alpha 5$ LG1-3 domains support the stemness of human mesenchymal stem cells. *Exp Ther Med*. 2020;21(2).
doi: 10.3892/etm.2020.9597
 20. Kim JE, Seo HJ, Lee S, Jang JH. Evaluation of Stemness Maintenance Properties of the Recombinant Human Laminin $\alpha 2$ LG1-3 Domains in Human Mesenchymal Stem Cells. *Protein Pept Lett*. 2019;26(10):785-791.
doi: 10.2174/0929866526666190617091155
 21. Jang SH, Jang JH. FN9-10ELP, an ECM-Mimetic Fusion Protein, Protects Human Mesenchymal Stem Cells from Etoposide-Induced Senescence. *Int J Mol Sci*. 2025;26(18):9218.
doi: 10.3390/ijms26189218
 22. Oh SA, Lee HY, Lee JH, et al. Collagen Three-Dimensional Hydrogel Matrix Carrying Basic Fibroblast Growth Factor for the Cultivation of Mesenchymal Stem Cells and Osteogenic Differentiation. *Tissue Eng Part A*. 2012;18(9-10):1087-1100.
doi: 10.1089/ten.tea.2011.0360
 23. Kim SG, Lee DS, Lee S, Jang JH. Osteocalcin/fibronectin-functionalized collagen matrices for bone tissue engineering. *J Biomed Mater Res A*. 2014;103(6):2133-2140.
doi: 10.1002/jbm.a.35351
 24. Karnik SK, Wythe JD, Sorensen L, Brooke BS, Urness LD, Li DY. Elastin induces myofibrillogenesis via a specific domain, VGVAPG. *Matrix Biol*. 2003;22(5):409-425.
doi: 10.1016/s0945-053x(03)00076-3
 25. Despanie J, Dhandhukia JP, Hamm-Alvarez SF, MacKay JA. Elastin-like polypeptides: Therapeutic applications for an emerging class of nanomedicines. *J Control Release*. 2016;240:93-108.
doi: 10.1016/j.jconrel.2015.11.010
 26. Kim W, Thévenot J, Ibarboure E, Lecommandoux S, Chaikof EL. Self-Assembly of Thermally Responsive Amphiphilic Diblock Copolypeptides into Spherical Micellar Nanoparticles. *Angew Chem Int Ed Engl*. 2010;49(25):4257-4260.
doi: 10.1002/anie.201001356
 27. Jeong ES, Park BH, Lee S, Jang JH. Construction and Evaluation of Recombinant Chimeric Fibrillin and Elastin Fragment in Human Mesenchymal Stem Cells. *Protein Pept Lett*. 2022;29(2):176-183.
doi: 10.2174/0929866528666211207110043
 28. Rifkin DB. Latent Transforming Growth Factor- β (TGF- β) Binding Proteins: Orchestrators of TGF- β Availability. *J Biol Chem*. 2005;280(9):7409-7412.
doi: 10.1074/jbc.r400029200
 29. Nettles DL, Chilkoti A, Setton LA. Applications of elastin-like polypeptides in tissue engineering. *Adv Drug Deliv Rev*.

- 2010;62(15):1479-1485.
doi: 10.1016/j.addr.2010.04.002
30. Rosano GL, Ceccarelli EA. Recombinant protein expression in *Escherichia coli*: advances and challenges. *Front Microbiol.* 2014;5.
doi: 10.3389/fmicb.2014.00172
 31. Estes BT, Wu AW, Guilak F. Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. *Arthritis Rheum.* 2006;54(4):1222-1232.
doi: 10.1002/art.21779
 32. Koivunen E, Wang B, Ruoslahti E. Phage Libraries Displaying Cyclic Peptides with Different Ring Sizes: Ligand Specificities of the RGD-Directed Integrins. *Nat Biotechnol.* 1995;13(3):265-270.
doi: 10.1038/nbt0395-265
 33. Lang K, Chin JW. Cellular Incorporation of Unnatural Amino Acids and Bioorthogonal Labeling of Proteins. *Chem Rev.* 2014;114(9):4764-4806.
doi: 10.1021/cr400355w
 34. Heilshorn SC, Liu JC, Tirrell DA. Cell-Binding Domain Context Affects Cell Behavior on Engineered Proteins. *Biomacromolecules.* 2004;6(1):318-323.
doi: 10.1021/bm049627q
 35. Trabbic-Carlson K, Setton LA, Chilkoti A. Swelling and Mechanical Behaviors of Chemically Cross-Linked Hydrogels of Elastin-like Polypeptides. *Biomacromolecules.* 2003;4(3):572-580.
doi: 10.1021/bm025671z
 36. Wang H, Cai L, Paul A, Enejder A, Heilshorn SC. Hybrid Elastin-like Polypeptide–Polyethylene Glycol (ELP-PEG) Hydrogels with Improved Transparency and Independent Control of Matrix Mechanics and Cell Ligand Density. *Biomacromolecules.* 2014;15(9):3421-3428.
doi: 10.1021/bm500969d
 37. Nie T, Baldwin A, Yamaguchi N, Kiick KL. Production of heparin-functionalized hydrogels for the development of responsive and controlled growth factor delivery systems. *J Control Release.* 2007;122(3):287-296.
doi: 10.1016/j.jconrel.2007.04.019
 38. Kim SJ, Kim SJ, Hyun J, Kim HW, Jang JH. Regeneration of Non-Alcoholic Fatty Liver Cells Using Chimeric FGF21/HGFR: A Novel Therapeutic Approach. *Int J Mol Sci.* 2024;25(6):3092.
doi: 10.3390/ijms25063092
 39. Martino MM, Tortelli F, Mochizuki M, *et al.* Engineering the Growth Factor Microenvironment with Fibronectin Domains to Promote Wound and Bone Tissue Healing. *Sci Transl Med.* 2011;3(100).
doi: 10.1126/scitranslmed.3002614
 40. Martino MM, Briquez PS, Güç E, *et al.* Growth Factors Engineered for Super-Affinity to the Extracellular Matrix Enhance Tissue Healing. *Science.* 2014;343(6173):885-888.
doi: 10.1126/science.1247663
 41. Jumper J, Evans R, Pritzel A, *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature.* 2021;596(7873):583-589.
doi: 10.1038/s41586-021-03819-2
 42. Abramson J, Adler J, Dunger J, *et al.* Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature.* 2024;630(8016):493-500.
doi: 10.1038/s41586-024-07487-w
 43. Watson JL, Juergens D, Bennett NR, *et al.* De novo design of protein structure and function with RFdiffusion. *Nature.* 2023;620(7976):1089-1100.
doi: 10.1038/s41586-023-06415-8
 44. Morrison SJ, Spradling AC. Stem Cells and Niches: Mechanisms That Promote Stem Cell Maintenance throughout Life. *Cell.* 2008;132(4):598-611.
doi: 10.1016/j.cell.2008.01.038
 45. Murphy WL, McDevitt TC, Engler AJ. Materials as stem cell regulators. *Nat Mater.* 2014;13(6):547-557.
doi: 10.1038/nmat3937
 46. Discher DE, Mooney DJ, Zandstra PW. Growth Factors, Matrices, and Forces Combine and Control Stem Cells. *Science.* 2009;324(5935):1673-1677.
doi: 10.1126/science.1171643
 47. Hamill KJ, Kligys K, Hopkinson SB, Jones JCR. Laminin deposition in the extracellular matrix: a complex picture emerges. *J Cell Sci.* 2009;122(24):4409-4417.
doi: 10.1242/jcs.041095
 48. Jeon E, Yun YR, Kim HW, Jang JH. Engineering and application of collagen-binding fibroblast growth factor 2 for sustained release. *J Biomed Mater Res A.* 2013;102(1):1-7.
doi: 10.1002/jbm.a.34689
 49. Lee S, Lee DS, Choi I, Pham L, Jang JH. Design of an Osteoinductive Extracellular Fibronectin Matrix Protein for Bone Tissue Engineering. *Int J Mol Sci.* 2015;16(4):7672-7681.
doi: 10.3390/ijms16047672
 50. Park S, Lee J, Ahn KS, *et al.* Cyclic Stretch Promotes Cellular Reprogramming Process through Cytoskeletal-Nuclear Mechano-Coupling and Epigenetic Modification. *Adv Sci (Weinh).* 2023;10(32).
doi: 10.1002/advs.202370218

51. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix Elasticity Directs Stem Cell Lineage Specification. *Cell*. 2006;126(4):677-689.
doi: 10.1016/j.cell.2006.06.044
52. Dupont S, Morsut L, Aragona M, *et al.* Role of YAP/TAZ in mechanotransduction. *Nature*. 2011;474(7350):179-183.
doi: 10.1038/nature10137
53. Coppé JP, Desprez PY, Krtolica A, Campisi J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annu Rev Pathol*. 2010;5(1):99-118.
doi: 10.1146/annurev-pathol-121808-102144
54. Pham QP, Sharma U, Mikos AG. Electrospinning of Polymeric Nanofibers for Tissue Engineering Applications: A Review. *Tissue Eng*. 2006;12(5):1197-1211.
doi: 10.1089/ten.2006.12.1197
55. Kim J, Noh K, Yu H, Lee H, Jang J, Kim H. A Fibronectin Peptide-Coupled Biopolymer Nanofibrous Matrix to Speed Up Initial Cellular Events. *Adv Eng Mater*. 2010;12(4).
doi: 10.1002/adem.200980008
56. Kang MS, Lee JH, Song SJ, *et al.* Graphene oxide-functionalized nanofibre composite matrices to enhance differentiation of hippocampal neuronal cells. *Mater Adv*. 2020;1(9):3496-3506.
doi: 10.1039/d0ma00733a
57. Andrew MacKay J, Chen M, McDaniel JR, Liu W, Simnick AJ, Chilkoti A. Self-assembling chimeric polypeptide-doxorubicin conjugate nanoparticles that abolish tumours after a single injection. *Nat Mater*. 2009;8(12):993-999.
doi: 10.1038/nmat2569
58. Dashnyam K, Perez R, Lee E, *et al.* Hybrid scaffolds of gelatin-siloxane releasing stromal derived factor-1 effective for cell recruitment. *J Biomed Mater Res A*. 2013;102(6):1859-1867.
doi: 10.1002/jbm.a.34838
59. Yun YR, Jang JH. Recombinant stromal cell derived factor 1 protein promotes neurite outgrowth in PC 12 cells. *Mol Med Rep*. 2020;23(1).
doi: 10.3892/mmr.2020.11699
60. Park JM, Koak JY, Jang JH, *et al.* Osseointegration of anodized titanium implants coated with FGF-fibronectin fusion protein. *Int J Oral Maxillofac Implants*. 2006;21(6):859-866.
61. Lee BSL, Yang N, Santos M, *et al.* Truncated vascular endothelial cadherin enhances rapid endothelialization of small diameter synthetic vascular grafts. *Mater Today Adv*. 2022;14:100222.
doi: 10.1016/j.mtadv.2022.100222
62. Divakarla SK, Das T, Chatterjee C, *et al.* Antimicrobial and Anti-inflammatory Gallium-Defensin Surface Coatings for Implantable Devices. *ACS Appl Mater Interfaces*. 2022;14(7):9685-9696.
doi: 10.1021/acsami.1c19579
63. Khanal D, Lei Q, Pinget G, *et al.* The protein corona determines the cytotoxicity of nanodiamonds: implications of corona formation and its remodelling on nanodiamond applications in biomedical imaging and drug delivery. *Nanoscale Adv*. 2020;2(10):4798-4812.
doi: 10.1039/d0na00231c
64. Kim T, Kim M, Eltohamy M, Yun Y, Jang J, Kim H. Efficacy of mesoporous silica nanoparticles in delivering BMP-2 plasmid DNA for in vitro osteogenic stimulation of mesenchymal stem cells. *J Biomed Mater Res A*. 2012;101A(6):1651-1660.
doi: 10.1002/jbm.a.34466
65. Kim HS, Lee JH, Mandakhbayar N, *et al.* Therapeutic tissue regenerative nanohybrids self-assembled from bioactive inorganic core/chitosan shell nanounits. *Biomaterials*. 2021;274:120857.
doi: 10.1016/j.biomaterials.2021.120857
66. Hofer M, Lutolf MP. Engineering organoids. *Nat Rev Mater*. 2021;6(5):402-420.
doi: 10.1038/s41578-021-00279-y
67. Heinrich MA, Liu W, Jimenez A, *et al.* Bioprinting: 3D Bioprinting: from Benches to Translational Applications (Small 23/2019). *Small*. 2019;15(23).
doi: 10.1002/smll.201970126
68. Ingber DE. Human organs-on-chips for disease modelling, drug development and personalized medicine. *Nat Rev Genet*. 2022;23(8):467-491.
doi: 10.1038/s41576-022-00466-9
69. MacEwan SR, Chilkoti A. Applications of elastin-like polypeptides in drug delivery. *J Control Release*. 2014;190:314-330.
doi: 10.1016/j.jconrel.2014.06.028
70. Schellekens H. Factors influencing the immunogenicity of therapeutic proteins. *Nephrol Dial Transplant*. 2005;20(suppl_6):vi3-vi9.
doi: 10.1093/ndt/gfh1092
71. Baker MP, Jones TD. Identification and removal of immunogenicity in therapeutic proteins. *Curr Opin Drug Discov Devel*. 2007;10(2):219-227.
72. Hanenberg H, Xiao XL, Dilloo D, Hashino K, Kato I, Williams DA. Colocalization of retrovirus and target cells on specific fibronectin fragments increases genetic transduction of mammalian cells. *Nat Med*. 1996;2(8):876-882.
doi: 10.1038/nm0896-876