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Reconstructing the hair follicle pigmentary unit using organoids and bioprinting

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Abstract

Hair graying is a common feature of physiological aging and is typically evaluated by hair color, melanin content, or melanogenesis-related enzyme activity. However, growing evidence suggests that hair graying results from functional defects at multiple levels of the hair follicle pigmentary unit (HFPU). The HFPU comprises both the intrinsic melanocyte lineage and the extrinsic microenvironment that supports melanocyte function. Here, we review the application of emerging technologies, including organoids, biomaterials, bioprinting, and microphysiological systems, to HFPU reconstruction. Based on the biological functions directly demonstrated by different models, we define six ascending levels of evidence: pigment production, melanocyte differentiation, pigment transfer, progenitor cell maintenance, niche reconstruction, and durable or cycle-like regenerative capacity. Current three-dimensional models reproduce selected structural or cellular components of the HFPU, but remain limited in their ability to sustain melanocyte-lineage renewal, support coordinated pigment transfer, and restore pigmentary function after perturbation. Distinguishing transient hair darkening or short-term melanogenic activation from functional HFPU reconstruction gives a more rigorous evaluation of hair repigmentation models and the interventions tested using these models.

Keywords: Hair follicle pigmentary unit; Hair graying; Hair repigmentation; Melanocyte stem cells; Skin organoids; Bioprinting; Extracellular matrix; Mechanobiology

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1. Introduction

Hair graying is a visible manifestation of aging that reflects the progressive decline of the follicular pigment-renewal system rather than a reduction in melanin synthesis alone.[1-3] During the physiological hair cycle, entry into anagen activates melanocyte stem cells and progenitors located in the hair germ and bulge regions of the follicle. Their progeny migrate toward the hair bulb, differentiate into mature melanocytes, produce melanosomes, and transfer these pigment-containing organelles to adjacent hair matrix keratinocytes, thereby pigmenting the emerging hair shaft.[4-6] This cyclic process is coordinated by the hair follicle pigmentary unit (HFPU), which integrates melanocyte-lineage cells, melanosome-receiving epithelial cells, dermal papilla-associated mesenchymal cells, the extracellular matrix, and regulatory cues within the local microenvironment.[2,4,5] Effective pigmentation therefore requires more than the

presence of melanocytes or the detection of melanogenic markers. It depends on the maintenance and timely activation of the stem and progenitor reserve, the spatially appropriate maturation of bulb melanocytes, efficient melanosome biogenesis and transfer, and sustained support from the surrounding epithelial, mesenchymal, and matrix compartments. Disruption at any of these levels—including stem-cell depletion, aberrant lineage differentiation, mature melanocyte dysfunction, defective melanosome production or transfer, or loss of microenvironmental support—can compromise pigmentation of the newly formed hair shaft.[3,7,8]

Longitudinal quantitative analyses of individual human hairs have revealed that previously gray hair segments can regain pigmentation, while partial, spontaneous, and treatment-associated hair repigmentation has also been documented clinically.[9] These observations indicate that some follicles retain a degree of pigmentary recovery capacity. However, visible repigmentation may reflect enhanced activity of residual mature melanocytes or transient relief of local inhibitory signals, rather than restoration of the melanocyte progenitor reserve, pigment-transfer interface, follicular niche, or broader microenvironment.[3,9] Repigmentation studies should therefore assess not only whether pigmentation increases, but also whether the HFPU that sustains cyclical pigment renewal has been functionally reconstructed.[1-3] Conventional models can resolve selected processes involved in hair follicle pigmentation but rarely reconstruct the HFPU as an integrated and continuously renewable functional unit. Monolayer melanocyte cultures are useful for studying melanogenesis, yet they lack the epithelial, mesenchymal, and extracellular-matrix environment required for lineage maintenance and pigment transfer. *Ex vivo* hair follicles preserve native architecture and short-term pigmentary activity, but their limited culture duration restricts assessment of long-term maintenance, repeated renewal, and functional recovery after injury. Animal models enable investigation of hair-cycle regulation and melanocyte stem cell (McSC) behavior *in vivo*; however, species-specific differences in follicular structure, cycling, and pigmentation limit direct extrapolation to humans.[3,5] Three-dimensional models have therefore emerged as an important approach for reconstructing HFPU cellular composition, spatial organization, and sustained pigmentary function.[10-12]

The main advantage of organoid models lies in their preservation of multicellular interactions and tissue self-organization. Neonatal mouse skin cells undergo sequential aggregation, polarization, fusion, and tissue rearrangement to generate hair-bearing skin, indicating that follicle morphogenesis depends on dynamic tissue reorganization rather than the simple juxtaposition of epithelial and mesenchymal cells.[10] Human pluripotent stem cell-derived skin organoids can also form pigmented skin-like tissues containing hair follicles, melanocytes, sebaceous glands, and sensory neural components, providing an experimental platform for studying human follicular development, melanocyte-lineage integration, and multicompartment tissue formation.[11,13] Reconstituted skin organoids have further revealed the stage-specific nature of niche signaling. During early tissue assembly, fibroblast-derived COL6A3-CD44 signaling supports melanocyte maintenance and positioning, whereas during follicle formation, bulge-derived SEMA3C-NRP1 signaling promotes melanocyte recruitment and regenerative pigmentation.[14] These findings indicate that melanocyte-lineage function depends not only on the presence of relevant cells and signals, but also on when and where those signals are presented. Comparatively simple skin cyst-like models may likewise retain latent morphogenetic potential, suggesting that initial structure alone does not fully determine subsequent tissue-forming capacity.[15]

Unlike organoids, which rely primarily on self-organization, bioprinting improves control over the initial cellular and material configuration. Hair follicle germ-like units containing epithelial and mesenchymal cells can be deposited at predefined positions, allowing regulation of cell ratios, intercellular spacing, matrix composition, and geometric arrangement while reducing variability in the starting configuration among constructs.[12] This spatial control is particularly relevant to HFPU reconstruction because melanocyte progenitors, mature hair-bulb melanocytes, hair matrix keratinocytes, and dermal papilla-associated mesenchyme must occupy distinct but functionally connected compartments.

Printed architecture, however, defines only the starting point of tissue assembly. Subsequent functional maturation still depends on cell migration, lineage differentiation, extracellular matrix remodeling, and sustained epithelial-mesenchymal communication.[10,12] Developmental pathways such as Wnt may coordinate these processes, but their effects are jointly determined by cellular state, signal intensity, and timing.[6,12,16] Printing fidelity and the formation of follicle-like structures therefore demonstrate control over the initial configuration, but do not by themselves establish functional HFPU reconstruction. Such a conclusion requires evidence that melanocyte stem or progenitor populations are maintained, mature melanocytes are generated in the appropriate compartment, melanosomes are transferred to hair matrix keratinocytes, and pigmentary output persists or is restored after a defined

perturbation.[2,5,12,17]

Existing three-dimensional models thus reproduce different subsets of HFPU composition, organization, and function, but the terms "repigmentation" and "HFPU reconstruction" are not used consistently across studies. Visible pigmentation may reflect increased melanin synthesis by residual mature melanocytes, enhanced survival of existing pigment cells, or transient activation of melanogenic pathways. None of these results alone demonstrates restoration of the follicular pigment-renewal system. To address this ambiguity, this review evaluates organoids, biomaterial-based constructs, bioprinted models, and microphysiological systems according to the biological functions they restore rather than their structural complexity or manufacturing precision alone.[11,12,18]

We organize the available evidence into six progressively demanding levels: pigment production, melanocyte differentiation, functional pigment transfer, progenitor cell maintenance, niche reconstruction, and durable or cycle-like regenerative competence. These levels should not be interpreted simply as differences in model complexity. Instead, they represent distinct functional claims that require correspondingly different forms of validation.

Based on these criteria, functional HFPU reconstruction requires the coordinated maintenance of the melanocyte stem cell or progenitor reserve, replenishment of differentiated melanocytes in the hair bulb, productive melanosome transfer to hair matrix keratinocytes, and sustained or repeatable pigmentation of newly generated hair structures. Recovery after a defined stress or injury provides a particularly stringent test because it distinguishes a model containing active pigment-producing cells from one capable of renewing the melanocyte lineage and restoring pigmentary function. The following sections therefore examine the multilevel mechanisms of hair graying, the cellular, spatial, material, and temporal requirements for HFPU reconstruction, and the functional range and validation criteria of current three-dimensional models. Figure 1 distinguishes visible pigmentation from functional HFPU repigmentation. Visible pigmentation may result from transient activity of residual mature melanocytes, whereas functional HFPU reconstruction requires maintenance of the McSC/progenitor reserve, replenishment of differentiated bulb melanocytes, productive melanosome transfer to hair matrix keratinocytes, and durable or repeatable pigment renewal.

2. Hair graying reflects multilevel dysfunction of the HFPU

Hair graying can arise from functional defects at multiple levels of the HFPU, including impaired maintenance of McSCs and progenitors, depletion or dysfunction of mature melanocytes in the hair bulb, defective melanosome biogenesis or transfer, and loss of epithelial, mesenchymal, or extracellular-matrix support.[3,4,19] These defects may occur independently or accumulate within the same follicle, and their relative contributions may vary with age, genetic background, anatomical site, and disease context.[3,20] Similar gray-hair phenotypes may therefore result from distinct underlying abnormalities, such as failure of lineage maintenance, impaired melanocyte maturation, defective pigment transfer, or insufficient niche support. Figure 2 illustrates three functional states of the HFPU: a healthy unit with an intact melanocyte lineage and effective pigment transfer; a potentially reversible state in which residual progenitor and pigmentary capacity is retained; and a dysfunctional gray unit marked by depletion of the McSC/progenitor pool, loss of mature bulb melanocytes, and reduced melanosome transfer.[3,14,19,21]

Sustained pigmentation across successive hair cycles depends on the preservation of the McSC and progenitor pool and its continued capacity to replenish mature melanocytes in the hair bulb. Impaired McSC self-maintenance accelerates hair graying in mice and is associated with progressive depletion of the melanocyte lineage in aging human follicles. In Bcl2-deficient mice, McSCs undergo selective apoptosis upon entering quiescence, leading to premature exhaustion of the stem-cell pool.[7] Genotoxic stress disrupts McSC maintenance through a different mechanism. Rather than simply inducing cell death, it promotes aberrant differentiation, causing McSCs to exit the stem-cell state prematurely and lose their subsequent regenerative capacity.[8] McSC maintenance also depends on regulatory signals from the surrounding niche. Transforming growth factor beta (TGF-β) signaling contributes to the preservation of McSC quiescence and an undifferentiated state by suppressing MITF and its downstream melanogenic program.[22] Intravital imaging, lineage tracing, and single-cell analyses further indicate that McSCs are not confined to a fixed state or location. They can move between the hair follicle stem-cell and transit-amplifying compartments and reversibly transition between less and more differentiated states. With aging, however, an increasing proportion of McSCs becomes confined to follicular regions from which they

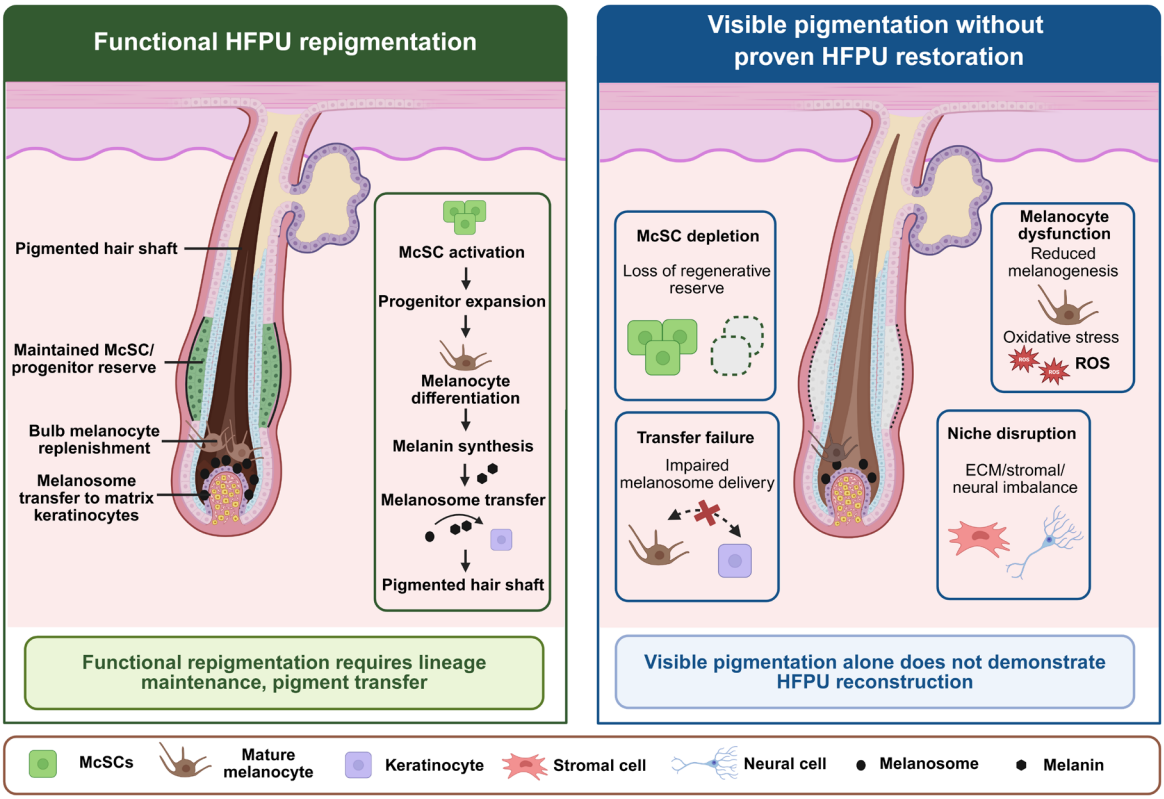

Figure 1. Functional repigmentation of the HFPU versus visible hair pigmentation without proven HFPU restoration. Schematic comparison of functional HFPU repigmentation and visible hair pigmentation without evidence of regenerative restoration. Left, functional repigmentation requires maintenance of the melanocyte stem cell (McSC)/progenitor reserve, McSC activation and progenitor expansion, differentiation into bulb melanocytes, melanin synthesis, and effective melanosome transfer to matrix keratinocytes. Together, these processes replenish bulb melanocytes, produce a pigmented hair shaft, and support pigmentation across successive hair cycles. Right, in contrast, visible hair pigmentation may result from transient melanogenesis or the activity of residual mature melanocytes and does not alone demonstrate restoration of the HFPU. McSC depletion, impaired melanosome transfer, melanocyte dysfunction associated with oxidative stress, or disruption of extracellular matrix, stromal, and neural niche signals may also prevent sustained repigmentation. Created in BioRender. Xie, Q. (2026) https://BioRender.com/fv41zog
Abbreviations: ECM, Extracellular matrix; HFPU, Hair follicle pigmentary unit; McSC, Melanocyte stem cell; ROS, Reactive oxygen species.

can no longer efficiently replenish mature melanocytes in the hair bulb.[21] Their reduced contribution to the pigmentation of newly formed hairs therefore reflects not only a decline in stem-cell number but also impaired state transitions and spatial redistribution. McSC dysfunction can thus be understood as the combined consequence of numerical depletion, premature differentiation, restricted state plasticity, and impaired spatial mobility. Evaluation of models for durable hair repigmentation should therefore establish not only whether McSC-like cells remain present, but also whether they retain the capacity to migrate, transition between states, generate mature melanocytes in the hair bulb, and repeatedly support pigmentation across successive hair cycles.

Even when the McSC reservoir is not completely exhausted, dysfunction of mature hair-bulb melanocytes or of the pigment-transfer process can directly reduce hair-shaft pigmentation. Mature melanocytes must sequentially complete melanin synthesis, melanosome maturation and intracellular transport, and melanosome transfer to adjacent hair matrix keratinocytes. Histological analyses of human follicles have shown that the transition from pigmented to gray and white hair is accompanied by a progressive reduction in melanocytes within both the hair bulb and the outer root sheath, indicating that loss of mature pigment-producing cells and depletion of the upstream lineage reservoir may occur concurrently.[19] Gray and white hairs accumulate hydrogen peroxide, exhibit reduced catalase activity and impaired methionine-sulfoxide repair, and show inhibition of tyrosinase function, supporting a contribution of oxidative stress to at least a subset of age-associated hair graying.[23] Studies of human hair follicles indicate that physiological hair graying

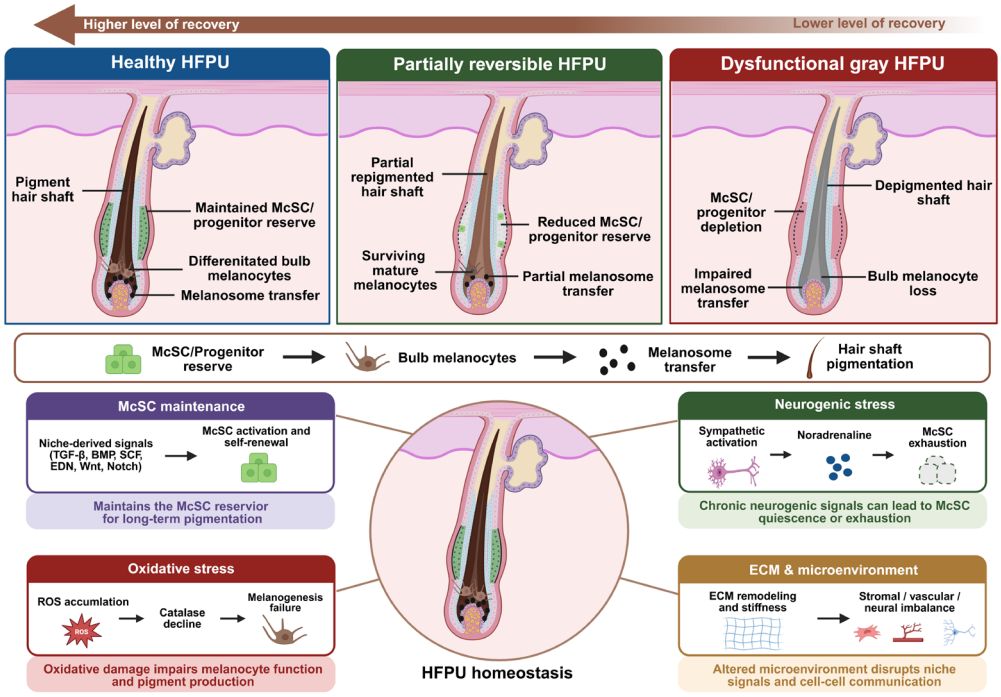

Figure 2. Hair graying reflects progressive failure of the HFPU regenerative hierarchy. The upper panels show healthy, partially reversible, and dysfunctional gray HFPUs along a gradient of decreasing recovery potential. In a healthy HFPU, the maintained melanocyte stem cell (McSC)/progenitor reserve replenishes differentiated bulb melanocytes, which transfer melanosomes to matrix keratinocytes and produce a pigmented hair shaft. A partially reversible HFPU retains a reduced McSC/progenitor reserve and surviving mature melanocytes. Partial melanosome transfer may therefore support limited hair repigmentation. In a dysfunctional gray HFPU, McSC/progenitor depletion, loss of bulb melanocytes, and impaired melanosome transfer result in a depigmented hair shaft. The central sequence summarizes the functional hierarchy from McSC maintenance and bulb melanocyte replenishment to melanosome transfer and hair shaft pigmentation. The lower panels show factors that maintain or disrupt HFPU homeostasis. Niche-derived signals support McSC maintenance and self-renewal, whereas oxidative stress impairs melanocyte function and pigment production. Chronic neurogenic stress may promote McSC quiescence or exhaustion, while extracellular matrix remodeling and stromal, vascular, or neural imbalance may disrupt niche signaling and cell-cell communication. Hair graying can therefore arise from failure at one or more levels of this hierarchy, and its reversibility depends on the persistence of the melanocyte lineage and a supportive follicular niche. Created in BioRender. Xie, Q. (2026) https://BioRender.com/majdg6m
Abbreviations: BMP, Bone morphogenetic protein; ECM, Extracellular matrix; EDN, Endothelin; HFPU, Hair follicle pigmentary unit; McSC, Melanocyte stem cell; ROS, Reactive oxygen species; SCF, Stem cell factor; TGF-β, Transforming growth factor β.

is generally accompanied by a reduction in melanocyte number and diminished melanogenic activity. However, whether defective melanosome transfer represents an independent pathogenic process, and the extent to which it contributes to hair graying, remain to be clarified.[3,20] HFPU models should therefore not assess pigmentary function only on the basis of total melanin content. Instead, mature melanocyte abundance, melanogenic capacity, and melanosome-transfer efficiency should be evaluated separately to distinguish defects at different functional levels.

Maintenance of the melanocyte lineage also depends on coordinated regulation by neural inputs, follicular epithelial and mesenchymal cells, and the extracellular matrix. In mice, acute stress activates sympathetic nerves innervating the McSC niche and rapidly increases local noradrenaline levels. This signal drives quiescent McSCs into excessive proliferation, differentiation, and migration, ultimately causing irreversible depletion of the stem cell reservoir and subsequent hair graying.[24] These findings demonstrate that sympathetic activation can directly disrupt McSC homeostasis and induce stress-associated hair graying. Skin organoid and transplantation studies have further revealed the spatial and temporal specificity of niche regulation. Fibroblast-derived collagen VI supports early melanocyte maintenance through COL6A3-CD44 signaling, whereas bulge-derived SEMA3C promotes melanocyte recruitment through NRP1-dependent regulation of cell positioning and supports hair pigmentation.[14]

Similar gray-hair phenotypes may therefore arise from defects at different functional levels, including depletion of the McSC reservoir, dysfunction of mature melanocytes, impaired melanosome biogenesis or transfer, and insufficient niche support. Defining these

mechanisms and their relative contributions is essential for accurately interpreting hair repigmentation results and for determining whether an intervention produces only a transient increase in pigmentation or restores an HFPU capable of sustained renewal.

3. Design and validation principles for functional HFPU reconstruction

The central objective of functional HFPU reconstruction is not to reproduce a static pigmentary phenotype, but to restore the tissue relationships and regenerative capacity required for sustained pigment production and renewal by the hair follicle. The presence of melanocytes, follicle-like structures, or visible pigmentation in an *in vitro* model demonstrates only that selected cellular components or short-term pigmentary activity have been established. It does not show that the melanocyte lineage can be continuously renewed, that an effective pigment-transfer interface has formed between mature melanocytes and hair matrix keratinocytes, or that pigmentary function can recover after injury. Based on the spatial organization and cyclical renewal of the native HFPU, functional reconstruction should be evaluated in four interrelated dimensions: maintenance of a renewable melanocyte lineage; establishment of the cellular organization and spatial adjacency required for pigment production and transfer; formation of a local niche that supports cell maintenance, assembly, and remodeling; and sustained pigmentary function or its recovery after perturbation. Figure 3 brings these requirements together and shows how cellular composition, spatial organization and functional interfaces, dynamic matrix regulation, temporal control of functional competence, and controlled manufacturing contribute to post-print self-organization, functional validation, and HFPU reconstruction.

3.1. Establishing a sustainably renewable melanocyte lineage

Functional HFPU reconstruction requires both immediate pigment production and a cellular reservoir capable of continuously replenishing mature melanocytes. Mature hair-bulb melanocytes synthesize melanin and export melanosomes, whereas pigment maintenance across successive hair cycles depends on McSCs and progenitors that repeatedly generate new differentiated melanocytes. Mouse studies have shown that McSCs are primarily located within the permanent portion of the hair follicle and that defective self-maintenance leads to depletion of the stem-cell reservoir and progressive hair graying.[7,25] Intravital imaging, lineage tracing, and single-cell analyses further demonstrated that McSCs can move between the bulge and hair-germ compartments and reversibly transition between stem-like and differentiated states. During aging, a proportion of these cells becomes trapped in locations from which they can no longer efficiently generate hair-bulb melanocytes, thereby losing their contribution to pigmentation of newly formed hairs.[21] Models containing only mature melanocytes therefore be suitable for assessing short-term melanogenesis, toxicity, or pharmacological responses, but they cannot demonstrate restoration of a pigment-cell reservoir or cyclical lineage renewal.

Maintenance of the melanocyte lineage also depends on organizational support from follicular epithelial and mesenchymal cells. Hair matrix keratinocytes receive melanosomes and incorporate pigment into the growing hair shaft, whereas epithelial cells within the permanent follicular compartment regulate McSC quiescence, activation, migration, and differentiation through direct contact and paracrine signaling. Dermal papilla cells contribute to the inductive and signaling environment of the hair bulb, but their biological function is determined not simply by their cellular designation; it is strongly influenced by anatomical origin, culture conditions, and passage history. Human dermal papilla cells progressively lose hair-inductive transcriptional features during two-dimensional expansion, whereas three-dimensional spheroid culture can partially restore their molecular identity and follicle-inductive capacity.[26] HFPU studies should therefore report the source, culture history, phenotypic state, and intended function of epithelial and mesenchymal cells.

The inclusion of additional cellular compartments should be determined by the biological question under investigation. Neural components are relevant to models of stress-induced McSC depletion, immune cells may be incorporated to reproduce inflammatory or injury-associated conditions, and endothelial or adipose-derived cells may be used to examine metabolic, angiocrine, or paracrine support. Such components are informative only when their role in the target process is mechanistically defined and experimentally testable. The cellular complexity of an HFPU model should therefore be judged by whether the melanocyte lineage and its essential supporting relationships have been accurately reconstructed, rather than by the number of cell types included.

3.2. Establishing the spatial architecture required for pigment transfer

HFPU function depends not only on cellular composition but also on whether melanocyte-lineage states are appropriately matched to their anatomical positions and tissue relationships.[2,27] In native follicles, McSCs and progenitors are mainly located within permanent

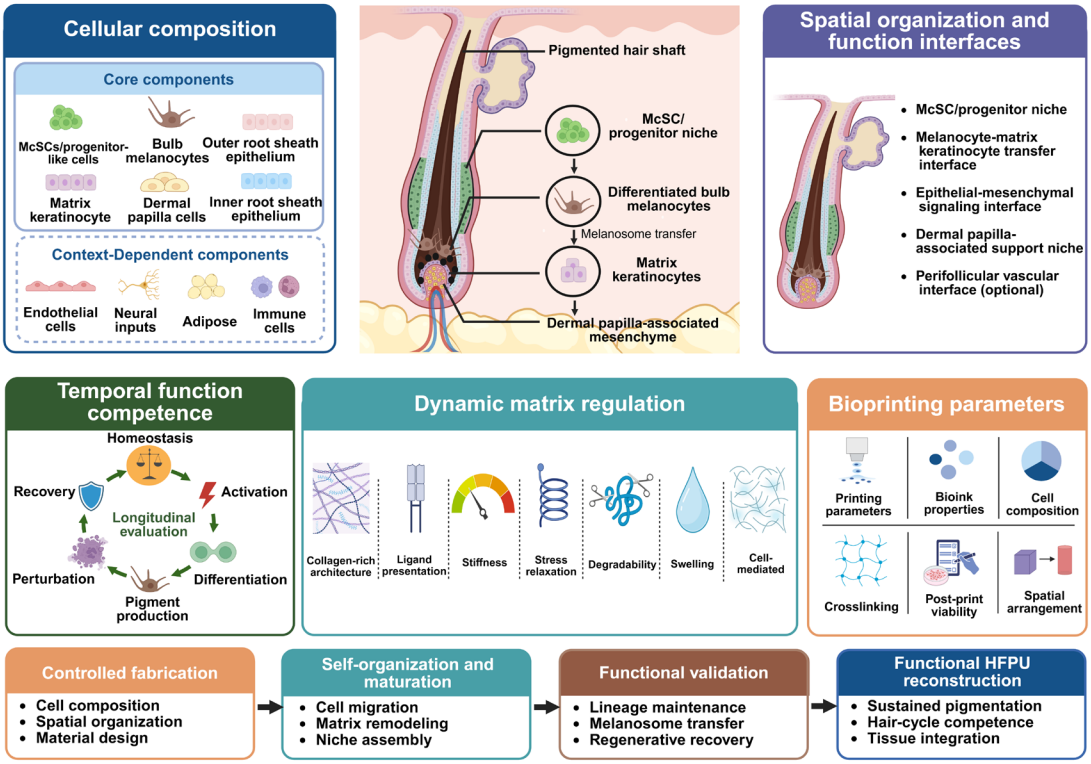

Figure 3. Design and validation requirements for functional HFPU reconstruction. The schematic summarizes five interrelated requirements for reconstructing a functional hair follicle pigmentary unit (HFPU): cellular composition, spatial organization and functional interfaces, temporal functional competence, dynamic matrix regulation, and bioprinting parameters. Core cellular components include melanocyte stem cell (McSC)/progenitor-like cells, differentiated bulb melanocytes, matrix keratinocytes, inner and outer root sheath epithelia, and dermal papilla cells. Endothelial, neural, adipose, and immune components may be included according to the biological question. Spatial organization should reproduce the McSC/progenitor niche, the melanosome-transfer interface between bulb melanocytes and matrix keratinocytes, epithelial-mesenchymal signaling interfaces, and dermal papilla-associated support. Perifollicular vascular interactions may also be incorporated. Temporal competence requires longitudinal assessment across homeostasis, activation, differentiation, pigment production, perturbation, and recovery. Matrix design should control collagen-rich architecture, ligand presentation, stiffness, stress relaxation, degradability, swelling, and cell-mediated remodeling. Bioprinting design includes printing parameters, bioink properties, cell composition, crosslinking, post-print viability, and spatial arrangement. Together, these inputs support controlled fabrication and guide cell migration, matrix remodeling, and niche assembly during post-print self-organization and maturation. Functional reconstruction should then be demonstrated by lineage maintenance, melanosome transfer, and regenerative recovery rather than pigment production alone. Models meeting these criteria may support sustained pigmentation, hair-cycle competence, and tissue integration. Created in BioRender. Xie, Q. (2026) https://BioRender.com/28uz8k5

Abbreviations: HFPU, Hair follicle pigmentary unit; McSC, Melanocyte stem cell.

compartments such as the bulge and secondary hair germ. Their progeny are activated during the hair cycle, migrate toward the bulb, and differentiate into mature melanocytes.[21,27] Mature melanocytes must subsequently establish close contact with hair matrix keratinocytes to transfer melanosomes to recipient cells and contribute to hair-shaft pigmentation.[2,28] Detection of melanocytes or melanocyte-associated markers in a three-dimensional model therefore confirms cellular presence, but does not demonstrate reconstruction of native spatial organization or productive pigment transfer.[17]

A functional HFPU model should reproduce at least three spatial relationships: a permanent-region-like niche that maintains McSCs and progenitors; a pigment-transfer interface between mature melanocytes and hair matrix keratinocytes; and an organized epithelial-mesenchymal relationship involving dermal papilla-associated cells.[2,14,27] Engineered human follicle studies have shown that defining the initial geometry of dermal papilla and epithelial cells using three-dimensional molds can promote follicular differentiation and hair growth after transplantation.[29] Hair follicle germ reconstruction studies further demonstrated that rearrangement of epithelial and mesenchymal stem cells together with their niches can generate follicles that undergo hair cycling and connect with the host epidermis, arrector pili muscles, and nerve

fibers after transplantation.[30] These findings establish the functional importance of spatial organization for follicle morphogenesis and tissue integration. However, because their principal endpoints were follicle formation and hair growth, they do not by themselves demonstrate restoration of melanocyte-lineage maintenance or melanosome transfer.[17,29,30]

Spatial proximity or fluorescence colocalization alone cannot establish functional pigment transfer, because the observed signal may reflect extracellular aggregation, attachment to the recipient-cell surface, or intracellular uptake.[28] Co-culture and live-cell imaging studies using normal human melanocytes and keratinocytes indicate that melanosome transfer comprises a sequence of donor-cell release, intercellular transmission, and uptake by recipient keratinocytes.[31,32]

HFPU models should therefore combine donor-specific melanosome labeling, live-cell tracking, three-dimensional imaging, and ultrastructural analysis to sequentially evaluate melanosome release, intercellular transfer, recipient-cell uptake, and intracellular localization.[31,32] Functional pigment transfer can be supported only when melanosomes of defined origin are detected within hair matrix keratinocytes and are subsequently shown to be incorporated into the developing hair shaft.[2,28] Likewise, expression of McSC-associated markers by dispersed cells does not demonstrate formation of a permanent-region-like niche capable of regulating quiescence, activation, and lineage replenishment.[21,27] Spatial reconstruction of the HFPU should therefore be evaluated by integrating cell state, anatomical position, and direct evidence of pigment transfer, rather than being inferred from marker expression or spatial proximity alone.[17,21]

3.3. Constructing a local niche for HFPU assembly and remodeling

The local HFPU niche comprises follicular epithelial cells, mesenchymal cells, extracellular matrix, and locally acting signals that collectively regulate melanocyte survival, migration, and state transitions during pigmentary-unit assembly and maintenance.[14,27] Time-dependent extracellular matrix processes, including stress relaxation, degradability, and cell-mediated remodeling, can also regulate cellular behavior.[33] Hydrogels with comparable initial elastic moduli, ligand densities, and degradation profiles may nevertheless induce different patterns of cell spreading, proliferation, and differentiation when their stress-relaxation kinetics differ.[33] The material design of HFPU models should therefore consider matrix composition, stiffness, stress relaxation, degradability, and cellular remodelability as distinct but interacting variables, rather than relying on any single parameter to evaluate niche function.

Skin organoid studies further indicate that the physical microenvironment directly participates in follicular tissue assembly. Neonatal mouse skin cells form hair-regenerative organoids through a multicellular self-organization process involving sequential epithelial and mesenchymal aggregation, polarization, migration, and tissue rearrangement.[10] During the early stage of this process, contractile forces generated by dermal cells promote mesenchymal-epithelial contact and regulate cell attachment and subsequent follicle regeneration through calcium signaling and epidermal PIEZO1.[34] During the later stage of tissue fusion, dermal stiffness and YAP1, together with Wnt signaling, matrix metalloproteinases, ROCK, laminin, and β1 integrin, coordinate cell migration and tissue rearrangement.[35] Follicle morphogenesis is therefore jointly regulated by biochemical signals and mechanical forces and depends on their coordinated action across space and time.[34,35]

Evidence for niche-dependent pigmentary reconstruction has also emerged from skin organoid models. During early organoid culture, fibroblast-derived COL6A3 supports melanocyte maintenance through CD44. During follicle morphogenesis after transplantation, bulge-derived SEMA3C promotes melanocyte recruitment to the bulge and matrix regions through NRP1- and microtubule-associated mechanisms.[14] Because these processes occur at different stages, they reveal that niche regulation of melanocytes is both cell source-dependent and temporally ordered.[14] Evaluation of an HFPU niche should therefore determine whether the local environment maintains the melanocyte lineage, supports migration and positioning, and promotes formation of the tissue relationships required for pigment transfer. Total melanin content or bulk material properties alone are insufficient for this purpose.[14,17]

In bioprinted models, the fabrication process itself modifies the cellular microenvironment. Nozzle geometry, extrusion pressure, shear stress, bioink rheology, and crosslinking conditions can influence post-print viability, phenotype, motility, and remodeling capacity.[36,37] Bioprinting of hair follicle germs enables automated arrangement of epithelial and mesenchymal microgels and uses cell-generated traction to promote collagen-microgel contraction and reorganization; after transplantation, these constructs can generate follicles and hair shafts.[12] However, the principal endpoints of that study were follicle induction and hair growth, without direct assessment of melanocyte reserve maintenance, melanosome transfer, or cyclical pigment renewal.[12] Printing accuracy therefore

characterizes only the initial manufacturing outcome. HFPU models must additionally demonstrate that cells can reorganize after fabrication and establish a niche that supports melanocyte maintenance, positioning, and pigment transfer.[12,17,36]

3.4. Validating Sustained pigmentary function and recovery after injury

The regenerative capacity of the HFPU cannot be inferred from pigmentation measured at a single time point, because hair coloration is coupled to cyclical McSC quiescence, activation, migration, and differentiation.[2,21,27] Prolonged survival of mature melanocytes or transient enhancement of melanogenesis can darken a construct without demonstrating continuous replenishment of pigment-producing cells.[2,21] Longitudinal analyses of individual human hairs have documented repigmentation of previously gray segments, but restoration of hair-shaft color alone does not establish concurrent recovery of the McSC reservoir or pigment-transfer interface.[9] HFPU models should therefore evaluate both the duration of pigmentary function and the capacity of the system to reconstruct that function after injury.[21,38]

Temporal analysis should distinguish the sequential processes of melanosome production, intracellular transport, release, uptake by recipient cells, and incorporation into the hair shaft.[28,32,39] Relevant quantitative endpoints may include melanosome production per melanocyte, the proportion of hair matrix keratinocytes that internalize melanosomes, pigment content per recipient cell, and the rate at which pigment is incorporated into the hair shaft.[32,39] Such measurements help distinguish changes in pigment production, transfer, and retention.

Compared with single-time-point assays, longitudinal comparison of three stages—stable function, reversible perturbation, and functional recovery—provides a more stringent test of HFPU regenerative competence. The model should first demonstrate stable maintenance of a McSC or progenitor reserve, mature melanocytes, productive melanosome transfer, and hair-shaft pigmentation under baseline conditions. A mechanistically defined and reversible intervention should then be applied to transiently impair pigment production or transfer. After withdrawal of the intervention, the model should be assessed for restoration of each functional component. In mice, transient inhibition of SCF–KIT signaling suppresses melanocyte proliferation and hair-shaft pigmentation during the current hair cycle, whereas retained McSCs can generate pigmented hair during the subsequent cycle.[38] Plucking-induced EDN3–EDNRB signaling can similarly activate McSCs and promote their proliferation, migration, and contribution to hair repigmentation.[40] Recovery of HFPU function should therefore not be inferred from renewed darkening alone; it should be accompanied by preservation of the progenitor reserve, regeneration of mature melanocytes, re-establishment of melanosome transfer, and renewed hair-shaft pigmentation.[38,40]

Microfluidic and organ-on-chip systems can control stimulus concentration, sequence, and duration while supporting longitudinal imaging, making them well-suited to the study of HFPU recovery after perturbation.[41] Current microfluidic follicle-organoid models have mainly improved the uniformity of follicle formation and transplantation efficiency, but have not yet demonstrated long-term renewal of the melanocyte lineage.[42] Transplanted follicle-germ organoids can integrate with host tissues and undergo repeated hair cycles, providing an *in vivo* reference for long-term functional assessment.[30] For the HFPU, however, renewed follicle growth or hair darkening remains insufficient evidence of pigmentary recovery. Restoration must include generation of mature melanocytes from a maintained progenitor compartment and renewed melanosome transfer to hair matrix keratinocytes.[2,17] The critical criterion for long-term HFPU function is therefore not simply how long pigmentation persists, but whether melanocyte replenishment and pigment transfer can be re-established after disruption.

3.5. Causal validation from pigmentary phenotype to functional reconstruction

Evaluation of HFPU reconstruction should progress from phenotypic observation to direct functional measurement and causal validation.[17] In hair follicle organoids, knockdown of genes associated with melanocyte maintenance and melanosome production, including *Bcl2* and *Mitf*, or genes associated with melanosome transport and transfer, including *MyoX*, *PAR2*, and *Rab11b*, increases the proportion of gray or white hair shafts. These interventions demonstrate that defects in pigment production and transfer can be functionally distinguished within the model.[17] Nevertheless, the model has not yet shown that an undifferentiated melanocyte reserve can be maintained over the long term and support repeated repigmentation across successive hair cycles.[17]

HFPU perturbation experiments should separately evaluate melanocyte survival and lineage state, melanosome production and transfer, and pigment deposition within the hair shaft.[2,17] Pharmacological studies should exclude cytotoxicity, altered proliferation, and off-target effects in non-target cell populations, and should be supported whenever possible by genetic intervention or rescue experiments.[17] For example, hair-shaft lightening after

inhibition of melanogenesis does not demonstrate defective melanosome transfer, whereas reduced pigmentation after inhibition of cell migration may result from impaired melanocyte survival or differentiation rather than defective positioning alone.[17,28,40]

For causal evaluation within an HFPU model, evidence may therefore be organized into five sequential tiers:

(i) The relevant cell populations and pigmentary components are present;
(ii) The cell state is matched to the appropriate anatomical location;
(iii) Melanosomes are shown to move from mature melanocytes into hair matrix keratinocytes and subsequently into the developing hair shaft;
(iv) Genetic or pharmacological perturbation demonstrates that the identified process is required for hair-shaft pigmentation;
(v) Melanocyte replenishment, pigment transfer, and hair-shaft pigmentation are re-established after withdrawal of the perturbation.[17,21,31,32,38]

The first two tiers support partial reconstruction of cellular composition and spatial organization. The third establishes functional pigment transfer, whereas the fourth and fifth provide stronger causal evidence for functional HFPU reconstruction.

4. *In vitro* models and technological strategies for HFPU reconstruction

Current three-dimensional models address distinct biological questions according to their specific strengths. Primary cell-based aggregates are suited to dissecting local cellular interactions; spatially guided assembly enables control over the initial positioning of epithelial and mesenchymal compartments; skin organoids reproduce multicellular lineage integration during development; and bioprinting improves the reproducibility of cellular, material, and tissue-unit arrangement.[11,12,29,43] Vascularized constructs, *in situ* bioprinting, and microphysiological systems further provide perfusion support, direct delivery within tissue defects, and temporally controlled stimulation, respectively.[18,44,45] These platforms are therefore complementary rather than interchangeable. Model selection should be determined by the biological objective, whether it is local mechanism dissection, spatial assembly, lineage development, or the long-term maintenance and recovery of pigmentary function.[12,17,18] Table 1 summarizes representative systems, HFPU-relevant features directly demonstrated, principal applications, major HFPU-specific limitations, and the interpretations supported by each platform class.

4.1. Primary cell-based and spatially guided models for dissecting hair follicle cell interactions

Primary cell-based microfollicles can be generated through temporally controlled coculture of outer root sheath keratinocytes, dermal papilla cells, and melanocytes, producing three-dimensional systems suitable for assessing follicle-associated markers and pharmacological responses.[43] Hair pigmentation organoids can further generate pigmented hair shafts *in vitro* and permit genetic interrogation of distinct processes contributing to hair color.[17] In this model, knockdown of Bcl2 and Mitf, which are associated with melanocyte maintenance and melanosome production, or of MyoX, PAR2, and Rab11b, which are involved in melanosome transport and transfer, increases the proportion of gray or white hair shafts.[17] Such reductionist models are therefore useful for rapidly testing defined cellular relationships and molecular processes. However, the presence of pigmented hair shafts alone does not demonstrate that a melanocyte stem cell or progenitor reservoir is maintained over the long term or remains capable of supporting cyclical pigment renewal.[17,43]

A central challenge for simplified hair follicle models is maintaining stable cellular states and region-specific tissue organization during prolonged culture.[26,43] Human dermal papilla cells progressively lose follicle-inductive transcriptional features during two-dimensional expansion, whereas three-dimensional spheroid culture can partially restore their molecular phenotype and hair-inductive capacity.[26] Spatially guided models seek to address this limitation by using molds, microwells, or preassembled spheroids to control the initial arrangement of epithelial and mesenchymal cells while retaining their capacity for subsequent reorganization.[29,46] For example, three-dimensional printed molds can position dermal papilla cell aggregates within elongated epithelial compartments, whereas coupled epithelial-dermal spheroids can gradually establish cellular polarity, basement membrane-like organization, and stable connections between the two tissue compartments.[29,46]

These studies indicate that initial geometry and epithelial-mesenchymal contact contribute to follicular morphogenesis. However, most current studies continue to use follicle-like structure formation or hair growth as their primary endpoints.[29,46] The available evidence does not establish that melanocyte stem cell or progenitor populations can be stably maintained and repeatedly support pigmentation over successive cycles. Spatial organization should therefore be considered a prerequisite for HFPU reconstruction, but not, by itself, evidence of durable hair repigmentation.

4.2. Skin organoids recapitulate follicular development and multilineage integration

Skin organoids reproduce the multicellular self-organization that accompanies hair follicle development. Neonatal mouse skin cells undergo sequential aggregation, polarization, fusion, and tissue rearrangement and can generate skin tissue with hair-regenerative capacity after transplantation.[10] Human pluripotent stem cell-derived skin organoids can form skin-like tissues containing pigmented hair follicles, sebaceous glands, melanocytes, Merkel cells, and sensory neural components.[11,13] Unlike spatially guided models, in which the positions of individual cellular compartments are predefined, skin organoids allow epithelial, mesenchymal, and neural crest-derived lineages to establish their spatial relationships progressively during development.[11,13] This self-organizing process preserves the dynamic features of lineage integration and tissue patterning, making these models suitable for investigating how melanocyte-lineage cells become incorporated into developing follicles and how multicellular tissue relationships emerge during follicle formation.

Reconstituted mouse skin organoids have also revealed stage-specific niche regulation of melanocyte maintenance and positioning.[14] During early culture, fibroblast-derived COL6A3 supports melanocyte maintenance through CD44-dependent signaling. After transplantation, as follicle formation begins, bulge-derived SEMA3C promotes melanocyte recruitment to the follicular niche through an NRP1-dependent mechanism associated with microtubule organization.[14] These findings indicate that melanocyte maintenance and positioning are temporally regulated by signals from distinct cellular sources at different stages of follicle development. Human pluripotent stem cell-derived skin organoids more closely resemble fetal or early developmental skin.[11,13] These models can recapitulate multilineage integration, melanocyte positioning, and pigmentation during follicle development.[11,13,14] However, their application to the study of gray-hair reversal requires further validation. Specifically, following a defined perturbation, it should be determined whether mature melanocytes can be replenished, pigment transfer can be re-established, and newly formed hair shafts can regain stable pigmentation.[14,17]

4.3. Structural control by bioprinting and post-printing tissue maturation

Bioprinting deposits cell-laden materials along predefined spatial paths, enabling control over cell ratios, tissue-unit dimensions, matrix composition, and the initial positions of individual cellular compartments.[12,47] Compared with manual assembly, its principal advantage is the reduction of structural variability among constructs, thereby providing more consistent starting conditions across experimental groups.[12,47] This consistency is particularly important for HFPU models because even small variations in cell spacing, compartment dimensions, or matrix distribution may alter epithelial-mesenchymal interactions and subsequently affect post-printing tissue maturation. However, completion of the printing process indicates only that the initial configuration has been established; it does not mean that functional tissue relationships have formed. Printed cells must still migrate and rearrange, establish epithelial-mesenchymal contacts, remodel the extracellular matrix, and exchange local signals before biologically meaningful tissue organization can emerge.[12,48] Retention of the predefined geometry therefore demonstrates spatial fidelity of fabrication, but cannot by itself establish that the intended cellular interfaces have matured or that the corresponding tissue functions have been achieved.[12,48]

The printing process itself can influence subsequent tissue formation. During extrusion and deposition, cells are exposed to pressure, shear stress, and transient deformation, and excessive shear can disrupt cell membranes and impair post-printing survival and proliferation.[37] In microvalve-based printing, the shear stress generated when droplets strike the substrate may exceed that experienced within the nozzle and can vary according to the nozzle-to-substrate distance.[49] HFPU bioprinting studies should therefore report the printing modality, nozzle dimensions, extrusion pressure, deposition speed, bioink properties, crosslinking conditions, and post-printing cell viability.[37,49] Model evaluation should further distinguish between fabrication fidelity, defined by the consistency of construct dimensions and cellular positioning, and tissue formation, defined by cell-state maintenance, post-printing reorganization, formation of functional tissue interfaces, and pigmentary activity.[12,17,48] A construct that retains its designed geometry but loses follicle-inductive mesenchymal identity or melanocyte-lineage competence cannot be considered biologically successful. Conversely, controlled post-printing contraction, migration, or matrix remodeling should not be regarded as a fabrication failure when these processes promote the formation of the intended tissue architecture.

4.4. Bioprinting of hair follicle germs and follicle-like structures

Bioprinting of hair follicle germs demonstrates how predefined epithelial-mesenchymal spatial relationships can guide subsequent post-printing tissue assembly. In this approach, collagen microgels containing epithelial or mesenchymal cells are deposited at adjacent positions. Cell-

generated traction forces then drive microgel contraction, cellular condensation, and further tissue rearrangement.[12] After transplantation into mice, these constructs form hair follicles and produce hair shafts, indicating that the initial spatial configuration and post-printing cellular self-organization can jointly support follicular regeneration.[12] However, existing studies have focused primarily on follicle induction and hair growth and have not directly assessed whether the constructs can maintain a stable melanocyte stem cell or progenitor reservoir over the long term, continuously replenish mature melanocytes, or sustain productive pigment transfer across multiple regenerative cycles.[12] At the tissue level, bioprinting can further incorporate follicle-associated cell populations into multilayered skin constructs.

Dermal papilla and endothelial cell spheroids surrounded by fibroblasts, keratinocytes, and melanocytes can generate structures with concentric cellular organization and early follicle-like features.[48] Gelatin-alginate and gelatin-hyaluronic acid hydrogels can also promote dermal papilla cell aggregation, preserve selected follicle-inductive characteristics, and support follicle-like structure formation *in vitro* or hair follicle formation after transplantation.[50,51] These models establish the feasibility of multicompartment fabrication and appendage-like tissue assembly. Nevertheless, they have not yet demonstrated an HFPU containing a stable bulge-like melanocyte reservoir and the capacity for cyclical repigmentation.[48,50,51]

4.5. Pigmentary and vascular support in bioprinted skin

Melanocyte-containing bioprinted skin permits controlled positioning of pigment cells within a stratified cutaneous structure. Constructs composed of fibroblasts, keratinocytes, and melanocytes can form a stratified epidermis with localized pigment deposition and can be used to investigate melanocyte distribution and epidermal pigmentation.[52] However, these systems lack the permanent region of the hair follicle, the specialized architecture of the hair bulb, and dermal papilla-associated mesenchyme, and therefore primarily reconstruct the epidermal melanin unit.[2,52] The presence of melanocytes, keratinocytes, and melanin deposits within these constructs indicates a degree of epidermal pigmentary function, but does not fully establish sustained melanocyte-lineage renewal, replenishment of mature melanocytes in the hair bulb, or pigmentation of newly formed hair shafts.[2,52]

Vascularized bioprinted skin further expands the cellular composition and functional scope of these models by incorporating endothelial cells, pericytes, and the potential for tissue perfusion. Constructs containing keratinocytes, fibroblasts, endothelial cells, and pericytes can form interconnected microvascular networks that anastomose with host vessels and become perfused after transplantation.[53] These models can be used to investigate oxygen and nutrient delivery, endothelial paracrine regulation, and the effects of perfusion on tissue maturation.[53] However, vascularization primarily improves nutrient supply and microenvironmental support and cannot substitute for the follicle-specific lineage hierarchy, regionalized spatial organization, or pigment transfer between hair bulb melanocytes and hair matrix keratinocytes required for HFPU function.[17,53] Vascularization should therefore be regarded as a supportive feature that increases tissue relevance, rather than as evidence of HFPU reconstruction in itself.

4.6. *In situ* bioprinting for wound repair and hair follicle regeneration

In situ bioprinting delivers cells and biomaterials directly to tissue defects, allowing spatial adaptation to irregular wound geometries and improving the precision of local cell and material deposition.[54,55] Unlike bioprinting strategies in which constructs are fabricated and matured *in vitro* before transplantation, *in situ* bioprinting does not require extensive preimplantation maturation but instead relies on the wound microenvironment to initiate and regulate subsequent tissue repair. In mouse wound models, robotic printing systems have been used to deposit epidermal stem cells, skin-derived precursor cells, and biomaterials such as Matrigel or gelatin methacryloyl (GelMA) directly into the wound bed. These approaches promote epidermal and dermal reconstruction, vascular formation, and the regeneration of skin appendages, including hair follicles and sebaceous glands.[54,55] These findings indicate that *in situ* bioprinting has the potential to support skin repair with appendage regeneration. However, current studies have focused primarily on wound closure and *de novo* hair follicle formation and have not yet established whether the regenerated follicles can maintain a long-term melanocyte stem cell or progenitor reservoir, sustain pigment transfer, or establish stable HFPU function.[54,55]

The appearance of newly formed mouse follicles does not by itself establish reconstruction of the follicular pigment lineage.[54,55] Application of *in situ* bioprinting to HFPU reconstruction would require additional evidence that regenerated follicles contain a maintainable melanocyte stem or progenitor reservoir, mature hair-bulb melanocytes, and an effective pigment-transfer interface with hair matrix keratinocytes.[2,17] *In situ* bioprinting therefore currently provides evidence of cellular and material delivery and appendage regeneration rather than complete HFPU reconstruction.[17,54,55]

4.7. Microphysiological systems for modeling dynamic environments and pigmentary recovery

Microphysiological systems use compartmentalized culture and dynamic perfusion to improve upon static culture conditions and extend the *ex vivo* maintenance of skin tissues or individual follicular units.[56] Existing platforms can accommodate engineered skin, skin explants, and isolated hair follicle units while using perfusion to regulate the culture environment and mechanical shear conditions.[56] Microfluidic devices have also enabled large-scale production of relatively uniform Janus-structured hair follicle germs, which retain epithelial and mesenchymal compartmentalization and support follicle regeneration after transplantation.[57] More recently, a hierarchical microfluidic organoid-on-chip platform was used to produce uniform hair follicle organoids and facilitate their intradermal delivery, although its principal endpoints remained organoid morphology, viability, transplantation, and hair growth rather than HFPU-specific pigment renewal.[42] These studies have primarily evaluated tissue viability, follicle formation, transplantation efficiency, or hair growth rather than long-term melanocyte-lineage renewal.[42,56,57] Combining microphysiological systems with organoid- or bioprinting-derived HFPU modules could enable controlled comparison of baseline function, reversible injury, and recovery after withdrawal of the stimulus. Dynamic assessment should extend beyond whether pigmentation increases or decreases after stimulation and should independently determine whether melanocyte stem or progenitor cells are retained, whether mature melanocytes are regenerated, and whether melanosome transfer and hair-shaft pigmentation are restored.[2,17]

The available microfluidic and organoid-on-chip studies have not yet demonstrated repeated renewal of an adult human melanocyte lineage together with functional melanosome transfer and recurrent hair-shaft repigmentation *in vitro*.[11,17,42,56,57] The recently developed pigmentation organoid model provides direct genetic access to melanosome production and transport, whereas recent microfluidic systems improve organoid uniformity and delivery; integrating these capabilities represents an important but still unrealized step toward a dynamic human HFPU platform.

4.8. Multiplatform integration and criteria for functional HFPU reconstruction

Each platform contributes to HFPU reconstruction at a different level. Simplified primary cell-based models are suitable for dissecting local cellular interactions; skin organoids enable observation of multilineage integration during development; spatially guided assembly tests the effects of initial epithelial-mesenchymal geometry; and bioprinting improves the reproducibility of cellular, material, and tissue-unit arrangement.[11,12,29,43] Vascularized constructs provide perfusion and endothelial support, *in situ* bioprinting delivers cells and materials directly into tissue defects, and microphysiological systems introduce temporal control through perfusion and programmable stimulation.[53,54,56] Figure 4 schematically illustrates the characteristic architecture and experimental role of six major three-dimensional platform classes discussed in this review. Multiplatform integration should therefore be organized around the biological process being tested rather than pursued solely to increase model complexity.[11,12,17,48]

Irrespective of the platform used, functional HFPU reconstruction ultimately requires resolution of three questions. First, can melanocyte stem and progenitor cells be maintained over time and continuously replenish mature melanocytes? Second, are melanosomes effectively transferred to hair matrix keratinocytes and incorporated into the developing hair shaft? Third, can pigmentary function be re-established after injury or withdrawal of a defined perturbation?[2,14,17] The presence of melanin or pigmented hair shafts demonstrates pigment formation, but functional HFPU reconstruction can be concluded only when lineage maintenance, productive melanosome transfer, and post-perturbation recovery are demonstrated together.[2,14,17]

5. Hierarchical evidence criteria for defining the extent of HFPU reconstruction

Visible pigmentation is the most intuitive result in HFPU models, but it is also the endpoint most susceptible to overinterpretation. Darkening of a construct may result from improved survival of mature melanocytes, transient enhancement of melanogenesis, increased melanosome maturation or transfer, local enrichment of pigment-producing cells, or attenuation of inhibitory signaling.[2,5,17] An increase in melanin content or the formation of pigmented hair shafts is therefore insufficient, by itself, to establish functional HFPU reconstruction. To align model evaluation with the biological functions directly demonstrated, Figure 5 organizes the available evidence into six levels: pigment production, melanocyte-lineage differentiation, functional melanosome transfer, McSC/progenitor maintenance, functional niche reconstruction, and durable regenerative competence.

Level 1: Pigment production

An observable increase in pigmentation of hair or tissue, increased melanin content, elevated tyrosinase activity, and enhanced Fontana-Masson staining can all serve as

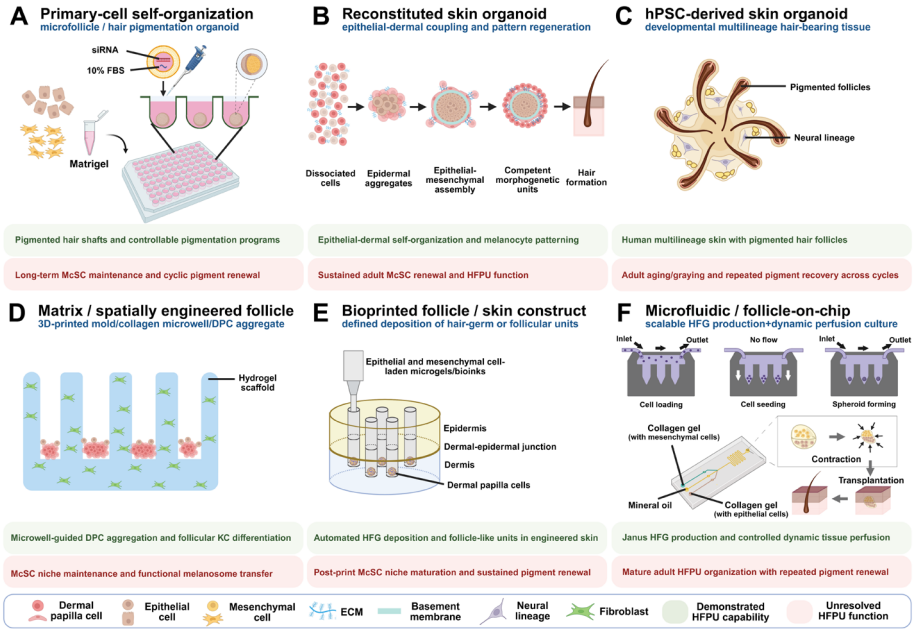

Figure 4. Representative three-dimensional platforms for modeling HFPU components and functions. Schematic comparison of six three-dimensional platforms with different cell sources, levels of tissue organization, and demonstrated HFPU-related functions. (A) Primary-cell self-organizing microfollicle and hair-pigmentation organoid models generate pigmented hair shafts and allow experimental control of pigmentation, but long-term melanocyte stem cell (McSC) maintenance and cyclic pigment renewal remain unresolved. (B) Reconstituted skin organoids reproduce epithelial-dermal self-organization, melanocyte patterning, and hair formation, whereas sustained adult McSC renewal and HFPU function have not been established. (C) Human pluripotent stem cell-derived skin organoids form multilineage skin containing pigmented hair follicles and neural components but do not yet reproduce adult aging, graying, or repeated pigment recovery across hair cycles. (D) Matrix- and spatially engineered systems use printed molds, collagen microwells, or dermal papilla cell aggregates to control follicular organization and keratinocyte differentiation. Maintenance of the McSC niche and functional melanosome transfer require further validation. (E) Bioprinting enables defined deposition of hair follicle germs or follicular units within engineered skin, but post-print McSC niche maturation and sustained pigment renewal remain uncertain. (F) Microfluidic and follicle-on-chip systems support scalable hair follicle germ production and dynamic perfusion culture, although mature adult HFPU organization and repeated pigment renewal have not been demonstrated. Green boxes indicate HFPU-related capabilities directly shown in representative studies. Red boxes indicate functions that remain unverified or incompletely reproduced. This comparison distinguishes the formation of pigmented follicles or follicle-like structures from functional HFPU reconstruction, which requires sustained McSC maintenance, melanosome transfer, and repeated pigment regeneration across hair cycles. Created in BioRender. Xie, Q. (2026) https://BioRender.com/emirh3w

Abbreviations: DPC: Dermal papilla cell; HFG: Hair follicle germ; HFPU: Hair follicle pigmentary unit; hPSC: Human pluripotent stem cell; KC: Keratinocyte; McSC: Melanocyte stem cell.

evidence of increased pigmentation within the model.[2,5,17] These measurements, however, do not identify the cellular basis of the pigmentary increase. The observed change may result from transient activation of residual mature melanocytes, increased melanocyte abundance, improved cell survival, or enhanced melanin synthesis, and therefore does not establish restoration of the complete melanocyte lineage. Level 1 evidence supports only an increase in pigment production or deposition and is insufficient to demonstrate melanocyte renewal, effective pigment transfer, or HFPU reconstruction.

Level 2: Melanocyte differentiation

Melanocyte-lineage differentiation should not be determined from the increased expression of a single marker but should be evaluated by integrating molecular features, cellular morphology, and spatial localization. Relevant lineage-associated markers include SOX10, MITF, DCT, TYR, TYRP1, PMEL, and MLANA, although the marker combination should correspond to the proposed differentiation stage.[4,5,58] Neural crest-derived precursors, melanocyte progenitors, and mature melanocytes, for example, exhibit distinct molecular profiles and occupy different tissue locations. Changes in a single marker reflect only one aspect of the differentiation process and cannot independently establish cellular identity or demonstrate continuous generation of mature melanocytes from stem or progenitor cells. Level 2 evidence can support the acquisition of specific melanocyte-lineage characteristics. Assessment of melanocyte differentiation should therefore integrate transcriptional or protein expression, cellular morphology, and localization within defined follicular

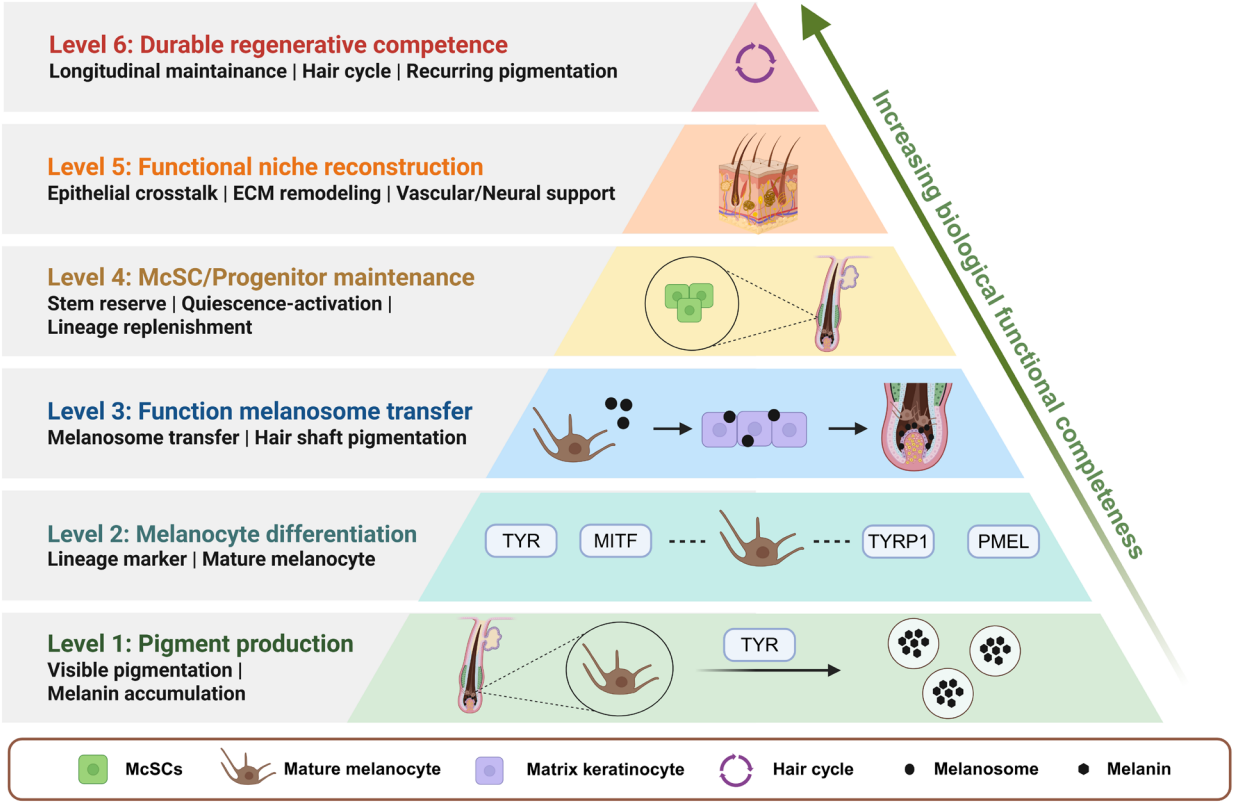

Figure 5. The HFPU reconstruction pyramid. Hierarchical framework for evaluating the extent of functional reconstruction in organoid and biofabricated pigmentary systems. The lower tiers represent essential but insufficient functions: Level 1, pigment production; Level 2, melanocyte differentiation; and Level 3, pigment transfer. The upper tiers capture regenerative competence: Level 4, maintenance of a melanocyte stem cell or progenitor reserve; Level 5, niche reconstruction, including epithelial–mesenchymal–melanocyte interactions and extracellular matrix organization; and Level 6, durable or cycle-like regenerative competence, defined by sustained or repeatable pigmentary function following time-dependent or stress-associated perturbations. Progression upward reflects increasingly stringent evidence, not a mandatory developmental sequence. Lower-tier outputs should not be interpreted as proof of higher-tier function. Created in BioRender. Xie, Q. (2026) https://BioRender.com/myoo5p0.
Abbreviations: ECM: Extracellular matrix; HFPU: Hair follicle pigmentary unit; McSC: Melanocyte stem cell.

compartments.

Level 3: Functional pigment transfer

For genuine hair-shaft pigmentation to occur, melanosomes produced by mature melanocytes must be transferred to hair matrix keratinocytes and subsequently incorporated into the developing hair shaft.[2,5,59] Spatial proximity between melanocytes and hair matrix keratinocytes, or pigment deposition around both cell populations, does not directly demonstrate productive transfer. Stronger evidence includes ultrastructural visualization, dynamic melanosome tracing, cell-type-resolved quantification of transfer, and direct detection of melanosomes or pigment incorporation within hair shaft-like structures.[17,59] The central distinction at this level is between a melanocyte that can produce pigment and a tissue system in which that pigment is received by the appropriate target cell and used for hair-shaft coloration.

Level 4: Maintenance of a melanocyte stem or progenitor reserve

A functional HFPU requires not only mature melanocytes but also a stem or progenitor population capable of continuously replenishing differentiated pigment cells from a permanent region- or bulge-like compartment.[7,58] Relevant endpoints include long-term persistence of precursor cells, appropriate spatial localization, maintenance of an undifferentiated state, and the capacity to generate mature melanocytes after activation. Persistent detection of precursor-like cells does not, however, establish self-renewal, and expression of stem-cell-associated markers alone does not demonstrate

regenerative function. More stringent evidence requires showing that the precursor population replenishes mature melanocytes after activation, that its selective depletion causes a corresponding loss of pigmentary function, and that lineage output can be re-established under recovery conditions.[6,8]

Level 5: Reconstruction of a functional local niche

Maintenance and renewal of the melanocyte lineage depend on organized spatial and functional interactions among follicular epithelial compartments, dermal papilla-associated mesenchyme, fibroblasts, and the extracellular matrix, with additional modulation by vascular, neural, metabolic, and immune signals.[6,60] The inclusion of multiple cell types or matrix components in a construct does not, by itself, demonstrate niche reconstruction. Niche function is established only when these components are shown to regulate melanocyte maintenance, activation, migration, differentiation, or pigment transfer. Compared with evidence based solely on coexistence or spatial proximity, selective depletion, signal blockade, spatial perturbation, and rescue experiments provide stronger causal evidence linking a niche component to melanocyte-lineage behavior.

Level 6: Durable or cycle-like regenerative competence

This level represents the highest evidentiary standard for functional HFPU reconstruction. It requires longitudinal demonstration that melanocyte-lineage organization, spatial architecture, and pigment transfer can be maintained over time or re-established after a defined perturbation. Because follicular pigmentation is tightly coupled to the hair cycle, a color change measured at a single time point cannot determine whether the system possesses sustained renewal capacity.[2] Oxidative stress, inflammation, metabolic disruption, precursor-cell depletion, or reversible inhibition of relevant signaling pathways can be used to impair pigmentary function, followed by longitudinal assessment of whether mature melanocyte replenishment, melanosome transfer, and hair-shaft pigmentation recover in a coordinated manner after withdrawal of the insult.[17,18]

Long-term maintenance of pigmentation under static culture conditions demonstrates relative stability of an existing phenotype, but does not independently establish cyclical renewal or regenerative recovery after injury. Current human organoid and microphysiological models primarily reproduce follicle formation, pigment production, or selected environmental responses and have not yet fully demonstrated the complete sequence of lineage renewal, functional pigment transfer, and post-perturbation repigmentation.[11,17,18]

Importantly, the evidence pyramid in Figure 5 represents increasing strength of biological inference rather than a fixed experimental sequence or obligatory developmental pathway. Increased pigmentation does not establish melanocyte-lineage reconstruction; marker expression does not establish cellular identity; spatial proximity does not establish melanosome transfer; and precursor-cell persistence does not establish self-renewal. Only when melanocyte-lineage replenishment, productive melanosome transfer, functional niche regulation, and recovery of pigmentation after perturbation are demonstrated together can the presence of pigment within a model be interpreted as evidence of functional HFPU reconstruction.[2,7,17,59,60]

6. Evidence stratification and standardized reporting for functional repigmentation

The six evidence levels outlined above can be consolidated into three categories of conclusions with increasing inferential strength (Figure 6). The first, visible pigmentation, denotes an increase in visible hair shaft pigmentation or melanin content but does not establish restoration of the melanocyte lineage or HFPU organization.[9,17] The second, transient pigmentary activation, refers to increased pigmentation accompanied by evidence of melanocyte differentiation or melanosome transfer, but without demonstration of a stable McSC/progenitor reserve, a functional regenerative niche, or sustained renewal capacity.[17,31,32] The third, functional HFPU reconstruction, should be reserved for models that demonstrate an organized melanocyte-lineage hierarchy, appropriate spatial integration, productive melanosome transfer, functional niche support, and durable or repeatable pigmentary regeneration.[7,14,21,38] Structurally simplified models remain useful for examining specific cellular interactions and molecular processes, but the conclusions drawn from them should be strictly limited to the functions directly tested. A local increase in pigmentation indicates only that pigment production or deposition has changed under the defined experimental conditions. In the absence of evidence for lineage maintenance, formation of key spatial interfaces, effective pigment transfer, and regenerative competence, such findings should not be extrapolated to functional reconstruction of the complete HFPU.[17,43] Table 2 summarizes the minimum evidence required for each functional level and outlines the additional validation needed to support stronger claims.

Reliable comparison across laboratories also depends on complete and standardized experimental reporting. At a minimum, studies should document cell source, donor background, passage history, culture duration, matrix

Table 1. Capabilities and limitations of organoid, bioprinted, and microphysiological models of the hair follicle pigmentary unit

Model platform	Representative systems	HFPU features reproduced	Primary application	Key limitation	Conclusion
Primary-cell aggregates and microfollicles	Human microfollicles; hair pigmentation organoids[17,43]	Short-term melanocyte survival and melanogenesis; limited epithelial–mesenchymal crosstalk; acute pigment transfer	Rapid screening of soluble factors, cell ratios, matrices, and drug responses	No stable bulge-like McSC compartment, adult polarity, or cycle-like behavior	Useful for short-term mechanisms, but not durable HFPU reconstruction
Molded and guided epithelial-mesenchymal assemblies	Biomimetic follicle models; coupled epidermal–dermal spheroids[29,46]	Controlled geometry, DP-like organization, polarity, and basement-membrane formation	Dissecting how tissue geometry and reciprocal cell contact shape follicular organization	Pigment-lineage renewal, sustained transfer, and repeated function are not directly tested	Models spatial organization rather than complete HFPU function
Self-organizing and hPSC-derived skin organoids	Newborn skin organoids; hPSC-derived hair-bearing skin; regenerative pigmentation organoids[10,11,13,14]	Developmental lineage integration, hair formation, matrix-dependent patterning, and pigmented appendages	Studying developmental assembly, positional cues, and tissue-level pigmentation	Predominantly fetal-like, with limited modeling of adult aging, perfusion, and cycling	Best suited to emergent multicellular organization during development
Direct cell-laden bioprinting	Gelatin/hyaluronic-acid and gelatin-alginate follicular skin constructs[50,51]	Programmed deposition of dissociated cells and matrix domains in filaments or droplets	Optimizing bioinks, deposition order, cell ratios, and post-print viability	Printed geometry may remodel during maturation; McSC maintenance and sustained pigment transfer remain unproven	Provides a reproducible starting architecture that still requires biological maturation
Spheroid, microgel, and follicle-germ bioprinting	Printed follicle germs; appendage-containing spheroid constructs; multicomponent artificial skin[12,48,65]	Preservation and placement of multicellular building blocks with defined epithelial–mesenchymal spacing	Testing aggregate size, local contacts, matrix composition, and appendage assembly	Adult human pigment-lineage hierarchy and repeatable function have not been established	Supports controlled multicellular assembly, but not full HFPU reconstruction
Pigmented and vascularized printed skin	Melanocyte-containing skin; vascularized and perfusable skin grafts; xeno-free vascularized grafts[44,52,53]	Epidermal pigment transfer, controlled melanocyte distribution, vascular networks, and perfusion support	Studying melanocyte-keratinocyte crosstalk, angiocrine signaling, transport, and material compatibility	Lacks follicular epithelium, the DP niche, and a defined McSC reserve	Provides pigmentary and vascular modules for later HFPU integration
In situ appendage-inclusive bioprinting	Robotic delivery of stem-cell-containing or GelMA-based bioinks in mouse wounds[54,55]	Conformal delivery of cells and matrix during appendage-inclusive wound repair	Evaluating automated deposition, wound conformity, and local appendage regeneration	Does not establish adult human HFPU identity or durable repigmentation	Demonstrates delivery and appendage-inclusive repair, not functional human HFPU reconstruction
Microphysiological and follicle-on-chip systems	Hair follicle organoid-on-chip; Skin-on-chip platforms; perfused vascularized bioprinted tissues[42,53,56,57]	Controlled gradients, perfusion, repeated exposure, and longitudinal imaging	Analyzing exposure history, stress-recovery responses, and vascular or metabolic regulation	No validated system combines mature human HFPU organization with repeated pigment renewal	Adds temporal and environmental control to organoid or printed modules

DP, dermal papilla; HFPU, hair follicle pigmentary unit; hPSC, human pluripotent stem cell; McSC, melanocyte stem cell. Model classes are distinguished by biological capability and fabrication strategy.

composition, and directly measured material properties. The spatial distribution of epithelial, mesenchymal, and melanocyte-lineage compartments should also be described, together with the experimental methods used to assess melanosome transfer and the maintenance of stem or progenitor populations.[21,26,29,31-33] This information influences not only model reproducibility, but also whether findings from different studies can be compared and interpreted appropriately.

Pigment-related readouts should likewise be interpreted in the context of cellular composition and spatial organization. Increased melanin content may result from changes in melanocyte abundance, differentiation state, or lineage composition and should therefore not be treated as an independent functional criterion. Markers associated with McSCs or melanocyte progenitors should also be evaluated in relation to their anatomical location, neighboring cellular interactions, and local tissue organization.[14,17,21] The expression of stem-cell-associated markers by dispersed cells does not demonstrate the formation of a permanent region-like niche capable of maintaining quiescence, regulating activation, and supporting lineage replenishment. Similarly, the detection of melanin or melanocyte markers cannot substitute for direct evidence of melanosome transfer and pigment incorporation into the developing hair shaft.[17,21,31,32]

Claims concerning matrix mechanics should be supported by direct measurements of relevant properties, including stiffness, viscoelasticity, stress relaxation, degradability, and rheological behavior, rather than inferred solely from polymer concentration, crosslinker content, or material formulation.[33,36] Even when materials have similar initial elastic moduli, degradation profiles, and adhesive ligand densities, differences in stress-relaxation kinetics can substantially alter cell spreading, proliferation, and differentiation. A single stiffness measurement is therefore insufficient to define the biological function of an engineered matrix.[33]

For models constructed using primary cells and engineered matrices, the information described above is particularly important because both cellular state and manufacturing conditions can alter structural and functional performance. Human dermal papilla cells progressively lose follicle-inductive transcriptional features and biological activity during two-dimensional expansion, whereas three-dimensional culture can partially restore their molecular phenotype and hair-inductive capacity.[26] Culture format and passage history should therefore not be treated as routine technical information, but as important variables that influence model performance and the interpretation of experimental findings.

Bioprinting introduces additional variability associated with material properties and the manufacturing process. Bioink rheology, printing pressure, and shear stress

Table 2. Evidence to distinguish increased pigmentation, transient pigment activation, and functional HFPU reconstruction

Category	Definition	Required evidence	Further evidence required
Increased pigmentation	Increased visible pigmentation or melanin production	Standardized color analysis, melanin quantification, Fontana–Masson staining, or tyrosinase activity	Melanocyte-lineage identity, spatial localization, and direct assessment of pigment transfer
Transient pigment activation	Active melanogenesis, improved survival of differentiated melanocytes, or pigment transfer without evidence of renewal	Increased pigmentation together with lineage markers, survival analysis, melanosome maturation, or transfer to recipient epithelium	Persistence of an McSC/progenitor reserve, organized niche interactions, and recovery after perturbation
Functional HFPU reconstruction	A spatially organized, stem-cell-supported pigmentary unit with sustained or repeatable function	Melanin production, differentiated melanocyte activity, productive transfer, precursor maintenance, and organized epithelial-mesenchymal-matrix support	Longitudinal persistence and recovery under defined cycle-like or stress-recovery conditions

Abbreviations: HFPU: Hair follicle pigmentary unit; McSC: Melanocyte stem cell.

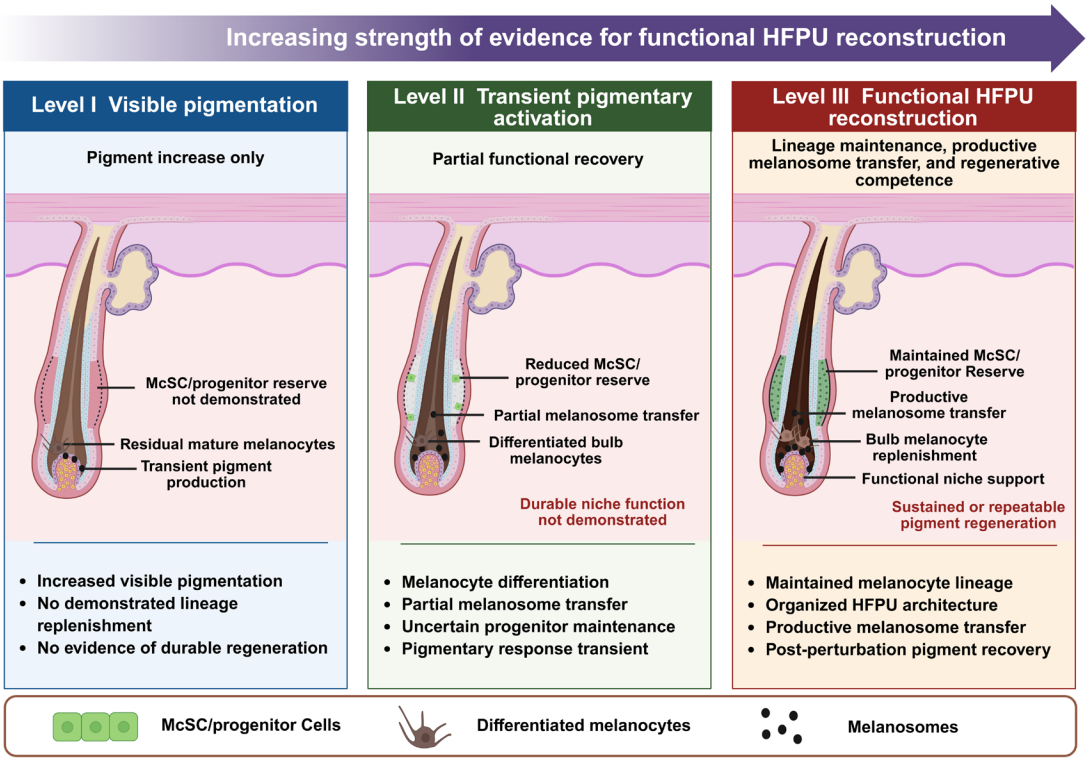

Figure 6. Evidence levels for distinguishing visible pigmentation, transient pigmentary activation, and functional HFPU reconstruction. The schematic classifies repigmentation results into three levels according to the strength of evidence for functional hair follicle pigmentary unit (HFPU) reconstruction. Level I, visible pigmentation, refers to increased hair pigmentation produced by residual mature melanocytes or transient pigment production. Without evidence of a maintained melanocyte stem cell (McSC)/progenitor reserve, lineage replenishment, or durable regeneration, this outcome demonstrates hair darkening only. Level II, transient pigmentary activation, includes melanocyte differentiation, partial melanosome transfer, and a possible reduction rather than complete loss of the McSC/progenitor reserve. These findings support partial functional recovery, but progenitor maintenance and durable niche function remain unproven, and the pigmentary response may be transient. Level III, functional HFPU reconstruction, requires maintenance of the melanocyte lineage, replenishment of bulb melanocytes, organized follicular architecture, functional niche support, and productive melanosome transfer. Sustained or repeatable pigment regeneration across hair cycles, including recovery after perturbation, provides evidence of regenerative competence. These levels indicate increasingly stringent evidence rather than an obligatory temporal sequence. Visible pigmentation alone is insufficient to establish functional reconstruction, which requires coordinated restoration of lineage maintenance, tissue organization, pigment transfer, and regenerative recovery. Created in BioRender. Xie, Q. (2026) https://BioRender.com/5riuirl

Abbreviations: HFPU: Hair follicle pigmentary unit; McSC: Melanocyte stem cell.

not only affect construct fidelity but also alter cellular integrity, phenotypic stability, proliferative capacity, and post-printing migration and tissue reorganization.[36,37] Manufacturing parameters should therefore be analyzed together with cellular state, spatial organization, and functional endpoints rather than reported separately from biological results. Integrating these factors within a unified assessment framework helps determine whether differences in model performance arise from technical variation during fabrication or reflect genuine biological changes. It also provides a clearer basis for distinguishing hair darkening, transient melanogenic activation, and functional HFPU reconstruction.[17,26,33,37] Complete, standardized, and traceable reporting of these variables is essential for improving the comparability and reproducibility of different experimental platforms and the reliability of the conclusions derived from them.

7. Next-generation HFPU models: Integrating bioprinting with tissue self-organization

The value of next-generation HFPU models lies not in their structural complexity, but in their ability to reproduce the key biological processes required to maintain hair follicle pigmentation.[10,12,35,61] A functionally informative model should preserve lineage continuity from McSCs or melanocyte progenitors to mature hair-bulb melanocytes, reconstruct the pigment-transfer interface between hair-bulb melanocytes and hair matrix keratinocytes, and maintain regulatory interactions between epithelial and

mesenchymal compartments. The model should also permit cell migration, matrix remodeling, and tissue maturation, while supporting longitudinal observation, selective perturbation, and assessment of functional recovery after injury or stress.[2,10,35,59] Together, these properties determine whether the model represents a static tissue structure or a functional HFPU capable of sustained maintenance and repair.[17,35,61]

Bioprinting and organoid self-organization address complementary aspects of model construction. Bioprinting can define the initial cellular distribution, compartment dimensions, and matrix environment, thereby providing relatively consistent starting conditions across experimental groups. Subsequent organoid self-organization allows cells to migrate, rearrange, and remodel the surrounding matrix, gradually establishing cellular contacts and functional interfaces that cannot be fully predetermined during printing.[10,12,35] The central design question is therefore not whether to choose bioprinting or self-organization, but which structures should be controlled during initial fabrication and which tissue relationships should be allowed to emerge dynamically during maturation.

The inclusion of vascular, neural, adipose, immune, or metabolic compartments should be guided by specific mechanistic questions. Communication between endothelial cells and the dermal papilla contributes to hair follicle regeneration and may be altered by age-associated vascular changes. Vascular components are therefore suitable for investigating the effects of perfusion, endothelial paracrine signaling, and the aging microenvironment on follicular function.[62] Neural components can be incorporated to model stress-induced HFPU destabilization. In mice, excessive sympathetic activation induces aberrant McSC differentiation and displacement from the niche, ultimately depleting the stem-cell reservoir.[24] Studies of human scalp follicles further suggest that signals derived from perifollicular adipose tissue can promote hair growth and pigmentation through HGF-associated pathways.[63] Printed vascular networks can provide additional control over tissue perfusion, oxygen supply, nutrient delivery, and the local metabolic environment.[44,62] However, vascular and other accessory compartments should be introduced to address clearly defined biological hypotheses rather than simply to increase model complexity. Each additional component should correspond to a specific scientific question, a defined HFPU process that it is expected to influence, and measurable functional endpoints. On this basis, a practical strategy is to use bioprinting to establish the initial cellular and material configuration, followed by organoid maturation and self-organization to generate the key interfaces and dynamic tissue relationships required for HFPU function.[10,12,35,48] This hybrid approach can improve the reproducibility of the initial construct while preserving the developmental plasticity required for cell migration, lineage differentiation, and matrix remodeling. Whether functional HFPU reconstruction has truly been achieved should ultimately be determined by evidence of McSC and progenitor maintenance, sustained replenishment of mature hair-bulb melanocytes, effective melanosome transfer, and recovery of pigmentary function after a defined perturbation.

In practice, epithelial cell aggregates, dermal papilla-like spheroids, melanocyte progenitors, and vascular microtissues could be deposited at defined ratios and distances within materials that support cell migration and matrix remodeling.[12,44,48] This modular approach can improve manufacturing consistency while preserving tissue self-organization and enabling the specific contribution of each compartment to HFPU assembly and function to be evaluated independently. Importantly, spatial relationships imposed during printing must be distinguished from those that emerge during maturation. A predefined position is biologically meaningful only when the cells retain the intended state and subsequently establish the relevant cellular contacts, paracrine interactions, or melanosome-transfer interfaces.[12,48,59]

For conceptual clarity, we propose three stages of HFPU engineering: modular reconstruction, coupled reconstruction, and durable reconstruction. Modular reconstruction establishes independently controllable units, such as a melanocyte-hair matrix keratinocyte pigment-transfer interface, a dermal papilla-like support module, or a defined matrix property, to test local cellular or material effects.[12,59] Coupled reconstruction integrates epithelial, mesenchymal, melanocyte-lineage, extracellular-matrix, and mechanism-relevant vascular or regulatory compartments to examine their functional interdependence.[44,48,62] Durable reconstruction further requires maintenance of a stem or progenitor reserve, reversible perturbation, and repeated recovery of pigmentation, thereby testing long-term or cycle-like regenerative competence.[2,3,17] These stages may be implemented using extrusion printing, droplet printing, spheroid or microgel deposition, vascular-channel fabrication, and *in situ* bioprinting; however, the fabrication method itself does not determine the biological level of HFPU reconstruction.[12,44,45,48] HFPU engineering should follow a minimum sufficient model principle. A simplified system should first be used to test a defined cellular relationship, after which additional compartments, material variables, and environmental signals should be

introduced only when supported by direct evidence.[35,61,64]

This stepwise construction strategy reduces the difficulty of attributing outcomes when multiple variables change simultaneously and enables the model to progress from local melanosome transfer to melanocyte-lineage renewal, niche regulation, and coordinated multicompartment regeneration.[59,61,64] Accordingly, validation criteria should become more stringent as the functional scope of the model expands. Early-stage models may focus on post-printing cell viability, melanocyte differentiation, maintenance of dermal papilla-associated characteristics, and melanosome transfer. More highly integrated models should additionally demonstrate progenitor maintenance, matrix remodeling, functional vascular perfusion, and recovery of pigmentary function after withdrawal of a reversible stressor.[3,12,17,44,48,59] The depth of validation should therefore correspond to the functions claimed for the model rather than being determined solely by its structural complexity. Reproducibility also depends on the coordinated reporting of manufacturing parameters and biological outcomes. Manufacturing information should include, at a minimum, printing modality, nozzle diameter or droplet size, deposition speed, printing pressure, temperature, crosslinking conditions, spatial fidelity, and post-printing culture duration. Biological information should encompass cellular composition and viability, phenotypic stability, spatial organization, matrix remodeling, and functional endpoints. Only by analyzing these two sets of variables together can differences in model performance be attributed to fabrication conditions, post-printing cellular adaptation, or genuine changes in HFPU function.

Biological variables should include cell source, donor background, passage number, post-printing viability, dermal papilla cell identity, melanocyte-lineage composition, epithelial maturation, matrix remodeling, and pigment-transfer efficiency.[12,43,48,59,65] Geometric fidelity indicates whether a construct was fabricated according to its intended dimensions and layout, but does not establish biological function. A geometrically accurate construct should still be considered unsuccessful if its dermal papilla-like mesenchyme loses follicle-inductive competence or its melanocyte stem or progenitor population cannot be maintained. Conversely, post-printing cell migration, tissue contraction, or matrix remodeling should be regarded as part of tissue maturation rather than fabrication error when these processes promote formation of the intended cellular interfaces and tissue architecture.[12,35,43] Claims concerning matrix mechanics should be supported by direct measurements of stiffness, viscoelasticity, stress relaxation, degradability, swelling, and rheological behavior, rather than inferred solely from polymer concentration, crosslinker content, or material formulation. This is particularly important for GelMA and related hydrogels, in which polymer-network entanglement and swelling kinetics can substantially alter time-dependent mechanical behavior even when the nominal formulations are similar.[66,67] Accordingly, a single stiffness measurement is insufficient to define matrix function; mechanical properties should be characterized under the actual culture conditions and interpreted together with cell-mediated matrix remodeling.

Models intended for adult hair-graying research should further treat aging-associated factors as experimentally defined, separable, and recombinable variables. Oxidative stress, inflammation, metabolic dysfunction, vascular impairment, neural signaling, and matrix remodeling should first be evaluated independently and then combined at defined doses and time points to distinguish additive from synergistic effects.[3,24,62] Patient-derived cells may help separate melanocyte lineage-intrinsic susceptibility from abnormalities in local tissue regulation, whereas human pluripotent stem cell-derived skin organoids are better suited to studying developmental lineage establishment.[3,11,68] Existing *in situ* bioprinting studies primarily establish conformal delivery of cells and materials and the regeneration of wound-associated hair follicles, rather than adult HFPU reconstruction or reversal of hair graying.[45,55,65] Greater human relevance will require scalp-relevant mesenchyme, well-defined donor and exposure histories, functional perfusion, and direct evidence of melanocyte replenishment, melanosome transfer, and hair shaft repigmentation after perturbation.[3,17,44,59,63] Broader studies in regenerative repair similarly indicate that skin repair models should not be evaluated solely on the basis of wound closure or barrier restoration, but should also assess higher-order regenerative outcomes, including the reconstruction of tissue architecture and recovery of skin appendage function.[69,70] Together, these principles define a staged workflow for next-generation HFPU engineering. As summarized in Figure 7, digital design and controlled manufacturing establish reproducible initial conditions; post-print self-organization generates functional cellular interfaces; perfusion and environmental control support tissue maturation; reversible challenge-and-recovery experiments test regenerative competence; and multimodal functional validation determines whether HFPU function is durable or repeatable.

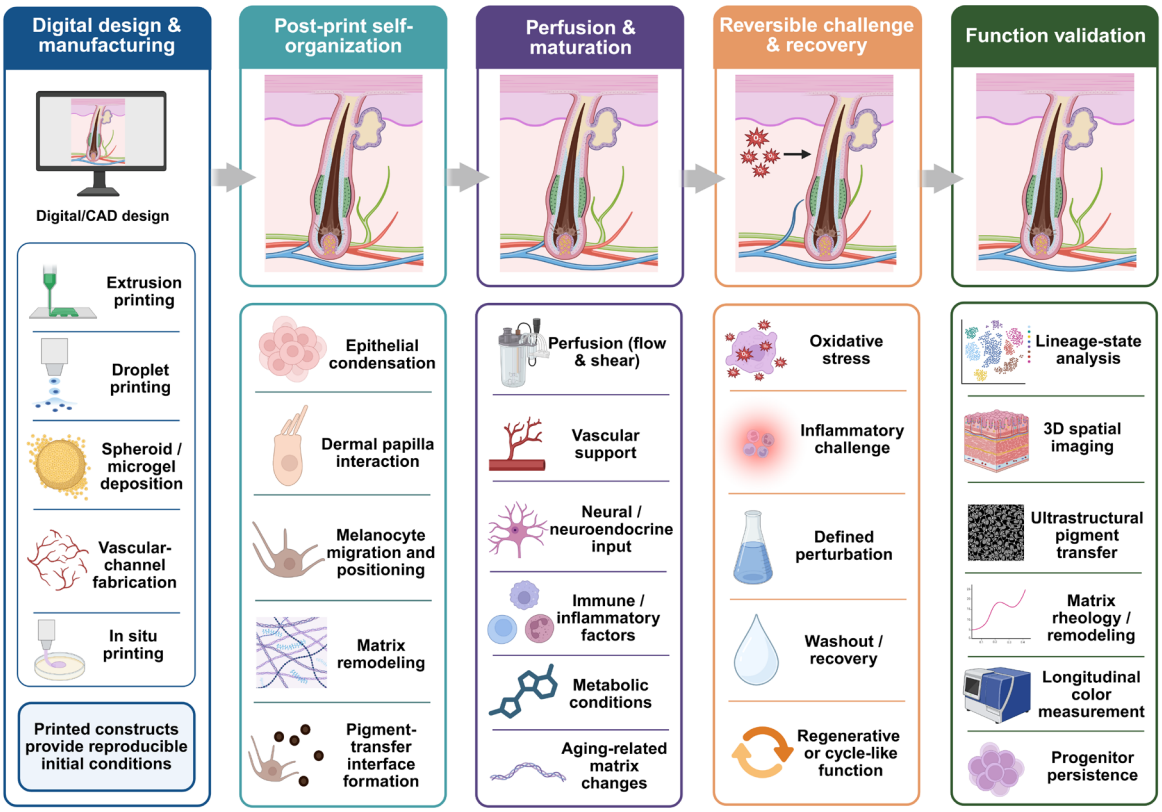

Figure 7. Engineering and validation for next-generation HFPU models. The schematic shows five connected steps for developing durable and human-relevant hair follicle pigmentary unit (HFPU) models. Digital design and manufacturing establish reproducible initial conditions through computer-aided design, extrusion or droplet printing, spheroid or microgel deposition, vascular-channel fabrication, and in situ printing. Post-print self-organization involves epithelial condensation, interaction with dermal papilla cells, melanocyte migration and positioning, matrix remodeling, and formation of the pigment-transfer interface. Perfusion and maturation introduce flow and shear, vascular support, neural and neuroendocrine inputs, immune or inflammatory factors, defined metabolic conditions, and aging-related matrix changes. Reversible challenge and recovery experiments expose the model to oxidative stress, inflammatory signals, or other defined perturbations, followed by washout and recovery. These experiments test whether pigmentation and follicular function can recover after disturbance and show regenerative or cycle-like behavior. Functional validation combines lineage-state analysis, three-dimensional spatial imaging, ultrastructural assessment of pigment transfer, measurement of matrix rheology and remodeling, longitudinal hair-color analysis, and evaluation of progenitor persistence. Functional HFPU reconstruction requires reproducible fabrication, appropriate self-organization and maturation, and demonstrated recovery after perturbation. The formation of a pigmented follicular structure alone is not sufficient to establish durable regenerative function. Created in BioRender. Xie, Q. (2026) https://BioRender.com/0wnyfli
Abbreviations: CAD: Computer-aided design; HFPU: Hair follicle pigmentary unit.

8. Conclusion

Hair graying is manifested by reduced or absent hair-shaft pigmentation, but it is not simply a consequence of diminished melanin synthesis. Similar visible phenotypes can arise from depletion of the melanocyte stem or progenitor reservoir, dysfunction of mature hair-bulb melanocytes, defective melanosome transfer, or loss of epithelial-mesenchymal and extracellular matrix support within the HFPU.[2,3,5,7] Hair pigmentation, increased melanin content, or induction of melanogenic markers demonstrates pigmentary activation but does not establish restoration of sustained HFPU renewal.[17,59]

Functional HFPU reconstruction requires a connected chain of evidence. A renewable melanocyte stem or progenitor population must be maintained within a permanent region- or bulge-like compartment, generate mature hair-bulb melanocytes, and support productive melanosome transfer to hair matrix keratinocytes and the developing hair shaft. In parallel, epithelial cells, dermal papilla-associated mesenchyme, fibroblasts, and extracellular matrix must form a local niche capable of regulating lineage behavior.[6,7,58,59] Longitudinal and reversible-perturbation experiments are then required to determine whether pigmentary function can be maintained or re-established after disruption. Only the combined

demonstration of lineage maintenance, functional pigment transfer, niche regulation, and post-perturbation recovery supports a conclusion of functional HFPU reconstruction.

Primary cell models, spatially guided assemblies, skin organoids, bioprinted constructs, and microphysiological systems provide complementary access to local mechanisms, developmental lineage integration, spatial control, and dynamic environmental regulation, but each currently reproduces only selected components of the HFPU.[10-12,18,43,48] Model value should therefore be judged by the biological question addressed and the strength of the functional evidence obtained, rather than by cell number, structural complexity, or geometric precision. The integration of bioprinting with organoid self-organization offers a rational route forward by combining reproducible initial conditions with the cellular rearrangement and matrix remodeling required for tissue maturation.[10,12,35]

Future HFPU engineering should proceed from minimum sufficient modules toward increasingly integrated systems, adding mesenchymal, vascular, neural, metabolic, and immune components only when they test defined mechanisms. Manufacturing parameters must be evaluated together with cell state, lineage composition, tissue maturation, matrix remodeling, and pigment-transfer function. The ultimate objective is not merely to generate a pigmented construct, but to establish a controllable, reproducible, and human-relevant regenerative system that maintains the melanocyte lineage, forms an effective pigment-transfer interface, and repeatedly restores hair-shaft pigmentation after perturbation.

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Conflict of interest

The authors declare that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors read and approved the final manuscript.

Availability of data

Not applicable.

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