

## REVIEW ARTICLE

# Advancements in hydrogel-based design and applications of hair follicle organoids

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## Abstract

Alopecia is globally recognized as a formidable therapeutic challenge, impacting both physiological health and psychological well-being. The crux of effective treatment lies in achieving *de novo* hair follicle neogenesis, a process that transcends merely stimulating existing follicles. Research into hair follicle organoids (HFOs) is advancing rapidly, transitioning from rudimentary self-assembly models toward high-fidelity, clinical application-driven paradigms. To provide a rigorous synthesis of the current landscape, this review conducted a systematic literature search across the Web of Science, PubMed, and Google Scholar databases. The search strategy utilized combinations of key terms, including “hair follicle organoid,” “alopecia,” “hydrogel,” and “3D bioprinting,” spanning the last 15 years. We comprehensively summarized the design principles and recent breakthroughs in HFO technology. First, the biological foundations of hair follicle development and the specific requirements of its inductive microenvironment were elucidated. Subsequently, we highlighted design strategies for functionalized hydrogels to simulate the hair follicle niche. This included a detailed discussion on modulating physicochemical properties and integrating advanced manufacturing technologies, such as three-dimensional bioprinting. Finally, the potential of HFOs in high-throughput drug screening and complex wound repair was assessed. By serving as a robust, human-relevant *in vitro* model, HFOs can significantly reduce reliance on animal testing and accelerate the discovery of hair-growth-promoting compounds. By providing both theoretical frameworks and technical insights, this review aims to support the development of high-performance hair follicle regeneration platforms and accelerate their transition from laboratory research to clinical translation.

**Keywords:** Alopecia; Hair follicle organoids; Hydrogel; Biofabrication; Three-dimensional bioprinting; Extracellular vesicles

## 1. Introduction

The etiology of alopecia is highly heterogeneous, involving a multitude of factors, such as genetics, senescence, hormonal imbalances, immune responses, psychological stress, and adverse drug reactions. According to statistics from the World Health Organization, approximately two billion individuals out of the global population of 8.25 billion are affected by alopecia, which profoundly impacts

patients' psychological well-being and social interactions.<sup>1-3</sup> The fundamental challenge in managing alopecia lies not merely in the activation of telogen-phase follicles to enhance hair density, but in the successful induction of *de novo* hair follicle neogenesis within alopecic regions. Conventional treatments, including pharmacological interventions (e.g., minoxidil and finasteride), injections, and laser therapy, often yield suboptimal results due to adverse side effects, prolonged treatment durations, and high recurrence

rates.<sup>4</sup> While hair transplantation surgery enables follicle regeneration in affected areas, physical compression during the procedure and chemical injury during follicle immersion in buffer solutions can compromise the quantity of viable donor follicles. Moreover, insufficient donor density and pre-existing scalp pathologies at the donor site further exacerbate the shortage of transplantable follicles,<sup>5,6</sup> rendering surgical intervention inadequate for a significant proportion of patients.

In recent years, the rapid advancement of regenerative medicine has provided novel perspectives for the treatment of alopecia, particularly through the *in vitro* construction of bioactive hair follicle organoids (HFOs) via tissue engineering. The hair follicle is recognized as a highly complex miniorgan, the development and cyclical growth of which are intrinsically dependent on epithelial–mesenchymal interaction (EMI).<sup>7–9</sup> While dermal papilla cells (DPCs) play a pivotal role in hair regeneration and activation of the growth cycle, human DPCs, unlike their murine counterparts, tend to lose their inductive capacity for hair follicle regeneration when maintained in monolayer cultures.<sup>10</sup> Consequently, the development of bioscaffolds that can closely mimic the native hair follicle and preserve the activity of human DPCs is considered essential. Furthermore, recapitulating complex spatiotemporal EMI in an *in vitro* environment remains a core challenge in engineering HFOs. Early research primarily focused on simple cell co-cultures. However, due to the lack of three-dimensional (3D) support and physical signaling, the induction of hair neogenesis *in vitro*—including the formation of mature follicular structures (such as the dermal papilla and bulge) and the elongation of long hair shafts—remains significantly challenging.<sup>11</sup> To address these limitations, hydrogels have emerged as a prominent delivery platform. The efficacy of hydrogels is predicated on creating an environment that closely mimics the natural extracellular matrix (ECM).<sup>12–16</sup> Specifically, gelatin methacryloyl (GelMA) hydrogels have become a focal point in HFO research owing to their inherent bioactivity and precise processability. GelMA not only retains the cell-adhesion motifs (e.g., arginylglycylaspartic acid sequences) and matrix metalloproteinase (MMP) degradation sites characteristic of collagen, but also enables the precise modulation of mechanical stiffness, pore size, and degradation rates through the adjustment of photocrosslinking parameters. This versatility provides the necessary physical confinement and biochemical signaling required for the growth and self-assembly of epithelial and mesenchymal cells.

This review aims to provide a comprehensive summary of the design principles and recent advancements in HFOs. Initially, the biological foundations of hair follicle development and the specific requirements of its

microenvironment are explored. Subsequently, we evaluate various hydrogel matrices—such as collagen, alginate, and synthetic polymers—detailing their respective strengths and limitations in supporting EMIs. Building on this comparative overview, we highlight the design principles of GelMA-based hydrogels, justified by their superior tunability and compatibility with advanced biofabrication. Finally, the application prospects of HFOs in drug screening and clinical wound repair are evaluated, alongside a discussion of current challenges and future directions in the field.

## 2. Biological basis of hair follicle development and principles of organoid construction

### 2.1. Key stages and spatiotemporal characteristics of hair follicle morphogenesis

The hair follicle is composed of epithelial and mesenchymal layers. The epithelial layer is composed of keratinocytes, while the mesenchymal layer encompasses the dermal sheath (DS) and the DPCs located at the follicular base. Cells within the dermal layers are recognized as highly specialized mesenchymal-derived fibroblasts, whereas the hair follicle stem cells (HFSCs) situated in the bulge region are responsible for the regenerative cycling of the follicle.<sup>17,18</sup> The primary objective of organoid technology is the induction of cellular self-assembly from disordered aggregation to ordered stratification (such as the formation of the inner and outer root sheaths) within 3D culture systems.<sup>19</sup> This process is highly dependent on spatial confinement, which is identified as a pivotal juncture for the functional intervention of bioscaffolds.

The morphogenesis of the hair follicle is recognized as a complex dynamic process, primarily driven by bidirectional signaling communication between epithelial and mesenchymal cells, a phenomenon known as EMI.<sup>20</sup> Through the secretion of signaling factors such as wntless-related integration site (Wnt),<sup>21</sup> hedgehog homolog 1,<sup>22</sup> and fibroblast growth factor (FGF),<sup>23</sup> the proliferation and differentiation of epithelial matrix cells are orchestrated by DPCs, ultimately leading to the formation of the hair shaft. In the context of organoid construction, the recapitulation of this heterotypic or interspecies communication is identified as a fundamental prerequisite for achieving hair follicle regeneration *in vitro*.

### 2.2. Fundamental principles of organoid construction

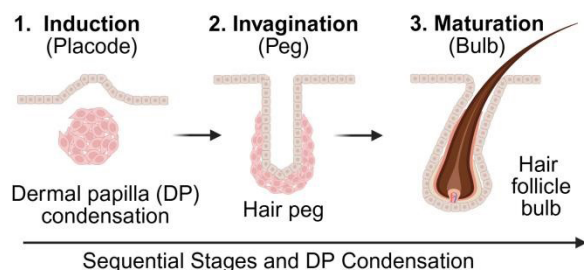
The construction of HFOs is recognized as having evolved from simple cellular mixtures to a sophisticated systemic-engineering endeavor.<sup>24</sup> Two fundamental strategies are primarily followed in the assembly of HFOs: self-

organization and niche mimicry. Self-organization involves the recapitulation of early embryonic states, wherein epithelial cells (e.g., keratinocytes) and mesenchymal cells (e.g., DPCs) are integrated at specific ratios in low-adhesion well plates. Spheres characterized by a core-shell structure are spontaneously formed by leveraging differences in intercellular affinity (Figure 1). For instance, it has been demonstrated that hair follicles, sebaceous glands, and adipocytes can be synergistically induced within 3D culture systems derived from homogeneous murine pluripotent stem cell populations by modulating transforming growth factor- $\beta$ , FGF, and bone morphogenetic protein signaling pathways. Through this methodology, the eight canonical stages of embryonic hair follicle development are recapitulated, enabling the generation of diverse follicular types, including guard, awl/auchene, and zigzag hairs.<sup>25</sup> Furthermore, ECM supplementation is utilized to enhance adhesion between epithelial and mesenchymal cells, thereby increasing the interfacial contact area and augmenting EMI.

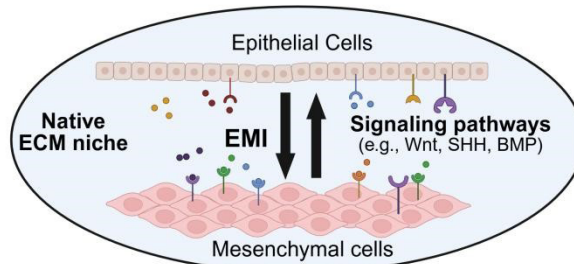
In contrast, the niche mimicry strategy is employed

to address the inherent lack of long-term structural stability and physical polarity observed in simple cellular aggregates, necessitating the introduction of functionalized bioscaffolds, such as Matrigel and GelMA. Physical support for cell adhesion is provided by these scaffolds, which further simulate the native cutaneous ECM through their mechanical stiffness and biochemical compositions, such as the incorporation of laminin or hyaluronic acid (HA).<sup>26,27</sup> The “trichogenic” inductive phenotype of DPCs is effectively maintained through this approach, while epithelial cell differentiation is guided toward specific lineages, thereby enhancing the induction rate and maturity of the organoids. Over the past three decades, a diverse array of natural biopolymers, including collagen, sodium alginate, gelatin, chitosan, silk fibroin, and HA, has been extensively explored. Additionally, significant contributions to the development of next-generation hair follicle culture technologies have been made using synthetic polymers, such as polyvinyl alcohol (PVA),<sup>28</sup> ethylene-vinyl alcohol copolymer,<sup>29</sup> poly(ethylene glycol) diacrylate,<sup>30,31</sup> and silicone.

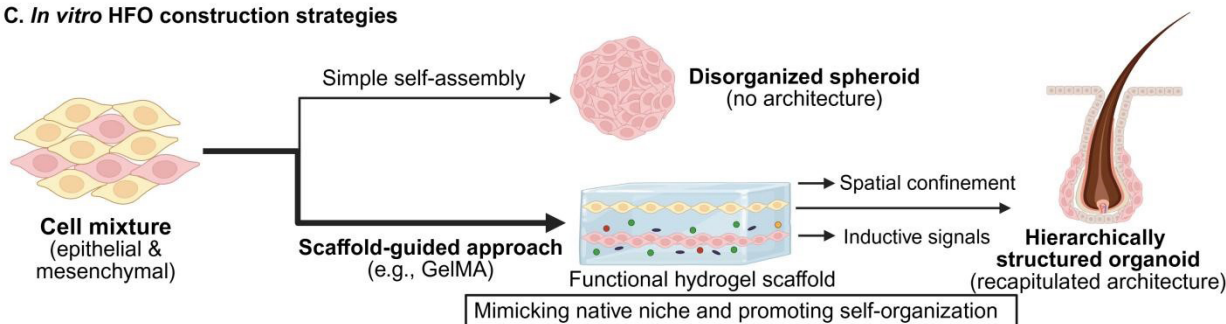
#### A. *In vivo* hair follicle morphogenesis



#### B. Core driving force: epithelial-mesenchymal interaction (EMI)



#### C. *In vitro* HFO construction strategies



**Figure 1.** Schematic illustration of natural hair follicle developmental biology and engineering principles for constructing HFOs. (A) *In vivo* hair follicle morphogenesis involves sequential stages: induction (placode), invagination (peg), and maturation (bulb), driven by the condensation of mesenchymal cells into the dermal papilla. (B) The core driving force is the reciprocal epithelial–mesenchymal interaction (EMI), mediated by complex signaling pathways within a specific native extracellular matrix (ECM) niche. (C) *In vitro* hair follicle organoid (HFO) construction strategies. While simple cell self-assembly often results in disorganized spheroids, scaffold-guided approaches (highlighted in the bottom path) utilize functional hydrogels (e.g., gelatin methacryloyl [GelMA]) to mimic the native niche’s mechanical and biochemical cues. These scaffolds provide necessary spatial confinement and inductive signals, promoting cellular self-organization into hierarchically structured organoids that recapitulate native follicle architecture. Figure created with BioRender.com. Qimanguli Saiding (2026) <https://BioRender.com/rzpcq8z>

### 3. Hair follicle scaffold design strategies

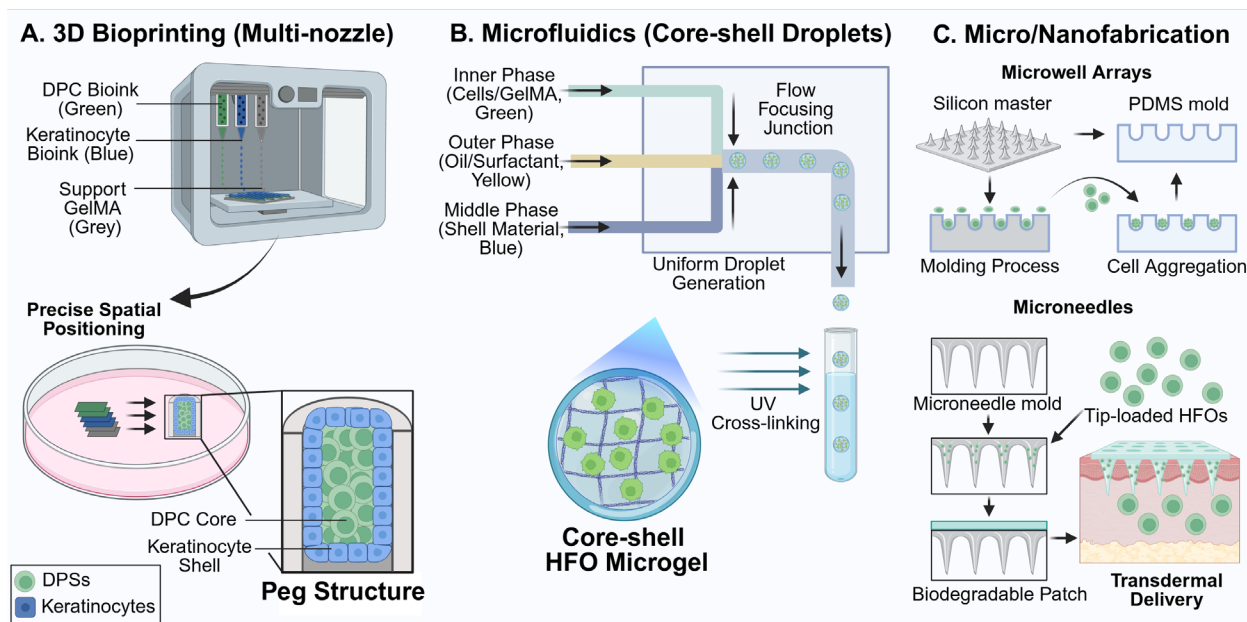
As discussed in Section 2, successfully engineering HFOs requires bioscaffolds that not only provide physical support but also closely mimic the native ECM to sustain EMI. To address these complex requirements, hydrogels have emerged as the most promising platform in tissue engineering. Their specific properties, such as degradation rates, mechanical strength, and surface chemistry, can be precisely customized. The synthesis of hydrogels is characterized by simplicity and exceptional tunability, enabling the individualized customization of physicochemical properties through the modification of substrates or reaction conditions (Figure 2).

#### 3.1. Comparative analysis of scaffolding materials

To successfully recapitulate the complex hair follicle niche *in vitro*, a diverse range of hydrogel matrices—spanning natural scaffolds to functionalized synthetic systems—have been extensively explored (Table 1). The selection of an appropriate biomaterial is critical, as it dictates the mechanical stability, biochemical signaling, and ultimate viability of the engineered organoid.

Natural proteins such as collagen were widely utilized

because they offer superior bioactivity and inherently possess cell-adhesion motifs (e.g., arginine–glycine–aspartic acid [RGD] sequences) that are critical for initial cell aggregation.<sup>32–35</sup> However, their poor mechanical stability remains a major bottleneck. During long-term 3D culture, these natural scaffolds are highly susceptible to cell-mediated contraction and rapid degradation, making it difficult to maintain the precise spatial architecture required for mature organoid development. Alternatively, natural polysaccharides like alginate<sup>36–39</sup> and chitosan<sup>40,41</sup> provide excellent biocompatibility and gentle gelation conditions suitable for cell encapsulation. Yet, their lack of intrinsic cell-binding sites fails to actively support EMI unless they undergo complex chemical modifications. Conversely, synthetic polymers (such as poly[ethylene glycol], PVA, and poly[lactic-co-glycolic acid]) offer high batch-to-batch consistency and exceptional tunability regarding mechanical stiffness and degradation rates. Despite these engineering advantages, their biologically inert nature hinders spontaneous cell migration and the complex signaling cross-talk necessary for hair follicle morphogenesis. To overcome the respective limitations of purely natural and purely synthetic materials, there is an urgent need for a hybrid biomaterial platform.



**Figure 2.** Schematic illustration comparing advanced biofabrication technologies for constructing hair follicle organoids (HFOs). (A) Three-dimensional (3D) bioprinting: A multi-nozzle extrusion-based printing system precisely deposits distinct bioinks (dermal papilla cell [DPC]-laden, keratinocyte-laden, and supporting gelatin methacryloyl [GelMA]) to reconstruct a biomimetic hair peg structure with defined spatial compartmentalization (DPC core and keratinocyte shell). (B) Microfluidic technology: A flow-focusing microfluidic device generates highly uniform core-shell droplets by manipulating multiphase fluid flows (inner cell phase, middle shell phase, and outer continuous oil phase), followed by ultraviolet (UV) cross-linking to form stable hair follicle organoid (HFO) microgels. (C) Micro/nanofabrication: utilizing soft lithography and molding techniques for two distinct applications: microwell arrays for high-throughput generation of uniform cell aggregates (top), and biodegradable microneedle patches loaded with HFOs at the tips for minimally invasive transdermal delivery (bottom). Figure created with BioRender.com. Qimanguli Saiding (2026) <https://BioRender.com/erwvg6r>



Among these, GelMA effectively bridges the gap between natural bioactivity and engineering precision by retaining essential RGD sequences for cell adhesion and MMP-sensitive motifs for cellular remodeling, while maintaining the tunable cross-linking density characteristic of synthetic resins. Consequently, the following sections mainly focus on GelMA-based design strategies, emphasizing how biomimetic microenvironments are optimized through parameter tuning and component hybridization to facilitate superior EMIs.

### 3.2. Precise regulation of physical properties

The maturation of HFOs is critically dependent on the physical confinement provided by the scaffold. By adjusting GelMA synthesis and cross-linking parameters, the growth environment for HFOs can be precisely customized.

Generally, higher concentrations (15–30%) yield

increased stiffness suitable for load-bearing tissues, whereas lower concentrations are considered more favorable for cell viability. It has been demonstrated that lower concentrations can prevent premature hydrogel formation and facilitate cellular aggregation; this is essential because intercellular contact between epithelial and mesenchymal cells is identified as a prerequisite for inducing EMI,<sup>11</sup> a phenomenon also reported in studies of inner ear and skin organoids. DPCs are recognized as being highly mechanosensitive during *in vitro* cultivation. By modulating the GelMA mass fraction (typically 5–10% w/v), the scaffold's storage modulus can be controlled within 1–10 kPa, matching the physiological stiffness of the human dermis.<sup>45</sup> Low-stiffness environments facilitate the maintenance of spherical DPC growth, subsequently upregulating the expression of inductive markers, such as alkaline phosphatase and Versican.

**Table 1. Comparison of hydrogel matrices for the development of hair follicle organoids**

Material class	Biocompatibility	Mechanical stability	Primary challenges	Clinical translation status
Natural proteins (e.g. collagen <sup>32-35</sup> )	Excellent: Contains RGD	Low: Susceptible to cell-mediated contraction and rapid degradation	Difficult to maintain a precise 3D architecture for long-term culture.	Widely FDA-approved, but less stable for complex organoids.
Natural polysaccharides (e.g., alginate, <sup>36-39</sup> chitosan <sup>40,41</sup> )	High: Non-toxic and gentle gelation; however, it lacks inherent cell-binding sites.	Moderate: Stable but lacks dynamic remodeling capabilities.	Requires chemical modification to support EMI.	Used in clinical trials for encapsulation; lacks specificity for hair regeneration.
Synthetic polymers (e.g., PEG, <sup>38</sup> PLGA, <sup>42</sup> PVA <sup>28</sup> )	Moderate: Inert; requires addition of growth factors or peptides.	High: Excellent tunability of stiffness and degradation rate.	Minimal biological instructive signals; difficult for cell migration.	Several FDA-approved platforms; high batch-to-batch consistency.
Semi-synthetic (e.g., GelMA <sup>12,26,43,44</sup> )	Excellent: Retains RGD/ MMPs.	High and tunable: Can be precisely controlled via UV/visible light and concentration	Standardization of photo-initiators and purification of raw materials.	Emerging: Subject to rigorous scrutiny regarding photo-initiator safety.

Abbreviations: 3D: Three-dimensional; EMI: Epithelial–mesenchymal interaction; FDA: Food and Drug Administration; GelMA: Gelatin methacryloyl; MMPs: Matrix metalloproteinases; PEG: Poly(ethylene glycol); PLGA: Poly(lactic-co-glycolic acid); PVA: Polyvinyl alcohol; RGD: Arginine–glycine–aspartic acid (cell adhesion peptide sequence); UV: Ultraviolet.

### 3.3. Composite systems for recapitulating the hair follicle microenvironment

Currently, Matrigel is the most widely used substrate in HFO research. The incorporation of Matrigel into DPC spheroids is found to significantly enhance follicular development and the efficiency of HFSC budding.<sup>11</sup> Furthermore, the uniform integration of mesenchymal stem cells (MSCs) and endothelial progenitor cells with low-concentration Matrigel is reported to activate the Wnt signaling pathway, thereby promoting hair follicle morphogenesis.<sup>46</sup> In a study by Kageyama *et al.*,<sup>11</sup> the primary constituents of Matrigel were analyzed, revealing that Type IV collagen, laminin-entactin complexes, and Type I collagen can efficiently induce hair shaft sprouting. Notably, Type I collagen—the most abundant ECM component *in vivo*—demonstrates an induction efficiency of up to 96%. However, the application of Type I collagen scaffolds is limited by their suboptimal mechanical properties.<sup>32</sup>

Additionally, the GelMA/HA composite system is identified as possessing significant potential for advanced applications. HA is recognized as a major ECM constituent of both the DS and the dermal papilla. The introduction of HA methacryloyl (HAMA) into the GelMA system not only improves the water retention of the scaffold but also facilitates the maintenance of DPC stemness and the secretion of trichogenic factors through CD44 receptor-mediated signaling pathways. It has been observed that HA can stimulate the activation of the Wnt pathway in MSCs via direct cellular contact, thereby promoting appropriate intercellular interactions and signaling during *in vitro* follicular development.<sup>27</sup> Kang *et al.*<sup>26</sup> developed an HA-incorporated bioink by mixing GelMA and HAMA at a 400:1 ratio—reflecting natural ECM proportions—to provide a native-like microenvironment for encapsulated DPC spheroids (Figure 3). Notably, under the influence of HA-modulated EMI, hair follicle units, encompassing both hair canals and inductive follicles, were observed to form spontaneously within the engineered scaffolds. These findings suggest that incorporating HA supports the expression of the intrinsic DPC phenotype. Although further *in vivo* efficacy assessments are required, this 3D-printed skin model is proposed as a novel approach for the biofabrication of hair-bearing skin.

Furthermore, the GelMA/alginate system is identified as another promising research avenue.<sup>37,38</sup> The incorporation of alginate is primarily utilized to modulate the rheological properties of the system. During 3D bioprinting of hair follicle arrays, GelMA/alginate hybrid inks exhibit excellent printability, enabling the fabrication of high-resolution microstructures that provide the spatial constraints necessary for site-specific positioning of follicles. The scope

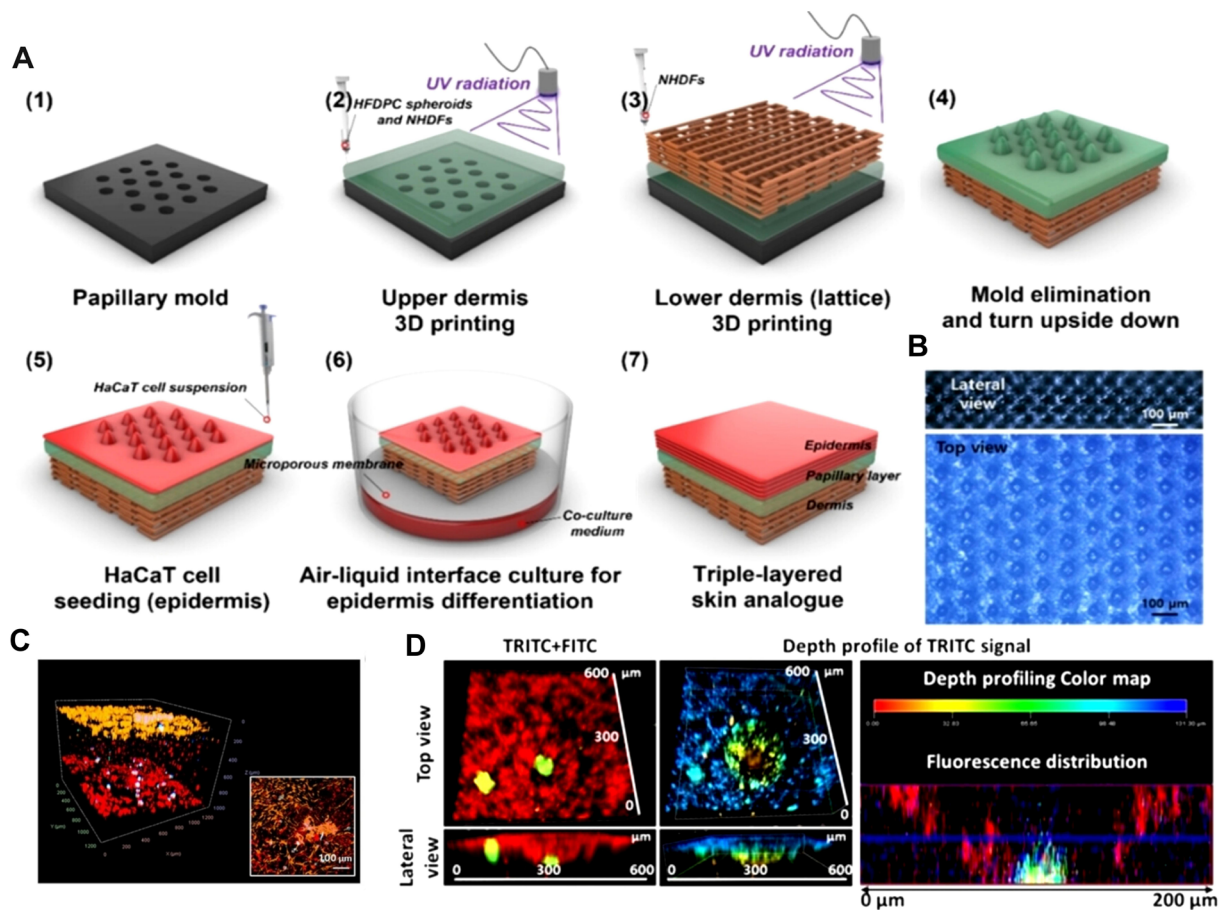
of future research on HFOs is significantly broadened by the inclusion of other functional additives. Chitosan is employed to enhance the interactions between the scaffold and epithelial cells, leveraging its inherent antibacterial properties and cationic nature.<sup>40,47–49</sup> Gold nanoparticles or iron oxide nanoparticles are utilized not only to reinforce the mechanical properties of the scaffold but also to facilitate *in vivo* imaging and tracking of the organoids. Furthermore, these nanoparticles are used to achieve the controlled release of localized signaling molecules through the photothermal effect.

### 3.4. Synchronizing degradation kinetics with hair follicle cyclicity

The hair follicle is recognized as a dynamic, cycling organ. By modulating the degree of substitution of GelMA, the degradation profile of the scaffold *in vivo* can be precisely controlled. Ideally, structural stability is maintained by the scaffold during the initial organoid budding phase (one to two weeks). However, as the hair bulb extends downward into the deep dermis or subcutaneous adipose tissue, the acceleration of the material's degradation kinetics is necessitated to prevent mechanical obstruction. Furthermore, at the conclusion of the growth phase, the induction of a stable telogen phase or the maintenance of the subsequent cycle should be facilitated by the released degradation products or encapsulated signaling molecules. In a study by Yao *et al.*,<sup>42</sup> a scaffold with controllable pore size and degradation rates was constructed using alginate/gelatin/alginate lyase hydrogels. This scaffold is designed to support the colonization and subsequent functional maturation of “hair follicular hanging drops.” Such a match ensures that sufficient mechanical support is provided during the critical stages of morphogenesis, while timely degradation accommodates the spatial requirements of follicular down-growth. Additionally, the concept of “artificial hair follicle seeding” was proposed by Ji *et al.*,<sup>50</sup> utilizing GelMA microspheres that serve not only as sustained-release vehicles for Wnt pathway activators but also exhibit degradation profiles synchronized with the time course of fibroblast reprogramming into DPCs (Figure 4). In summary, temporal control achieved through degradation kinetics is essential for the successful induction of HFOs.

## 4. Advanced manufacturing technologies for hair follicle organoids

While biochemical signals are provided by composite hydrogels, achieving a spatially ordered hair follicle arrangement depends on advanced manufacturing technologies (Table 2). Traditional organoid construction often relies on the spontaneous aggregation of cells in



**Figure 3.** Three-dimensional (3D) bioprinting process and characterization of a triple-layered skin analog with papillary microstructures. (A) Schematic of the 3D bioprinting and construction process. (B) Lateral and top views of the printed construct. Scale bar: 100 µm; magnification: 40×. (C) 3D fluorescence imaging. Scale bar: 100 µm; magnification: 40×. (D) Fluorescence distribution and depth profiling. Reprinted with permission from Ref.<sup>26</sup> Copyright © 2022 John Wiley and Sons Ltd.

Matrigel, a process characterized by inherent randomness and morphological heterogeneity. Through spatial micromanipulation of scaffold materials such as GelMA, the precise simulation of polarity, array formation, and the complex microenvironments of HFOs is facilitated.

#### 4.1. Three-dimensional bioprinting technology

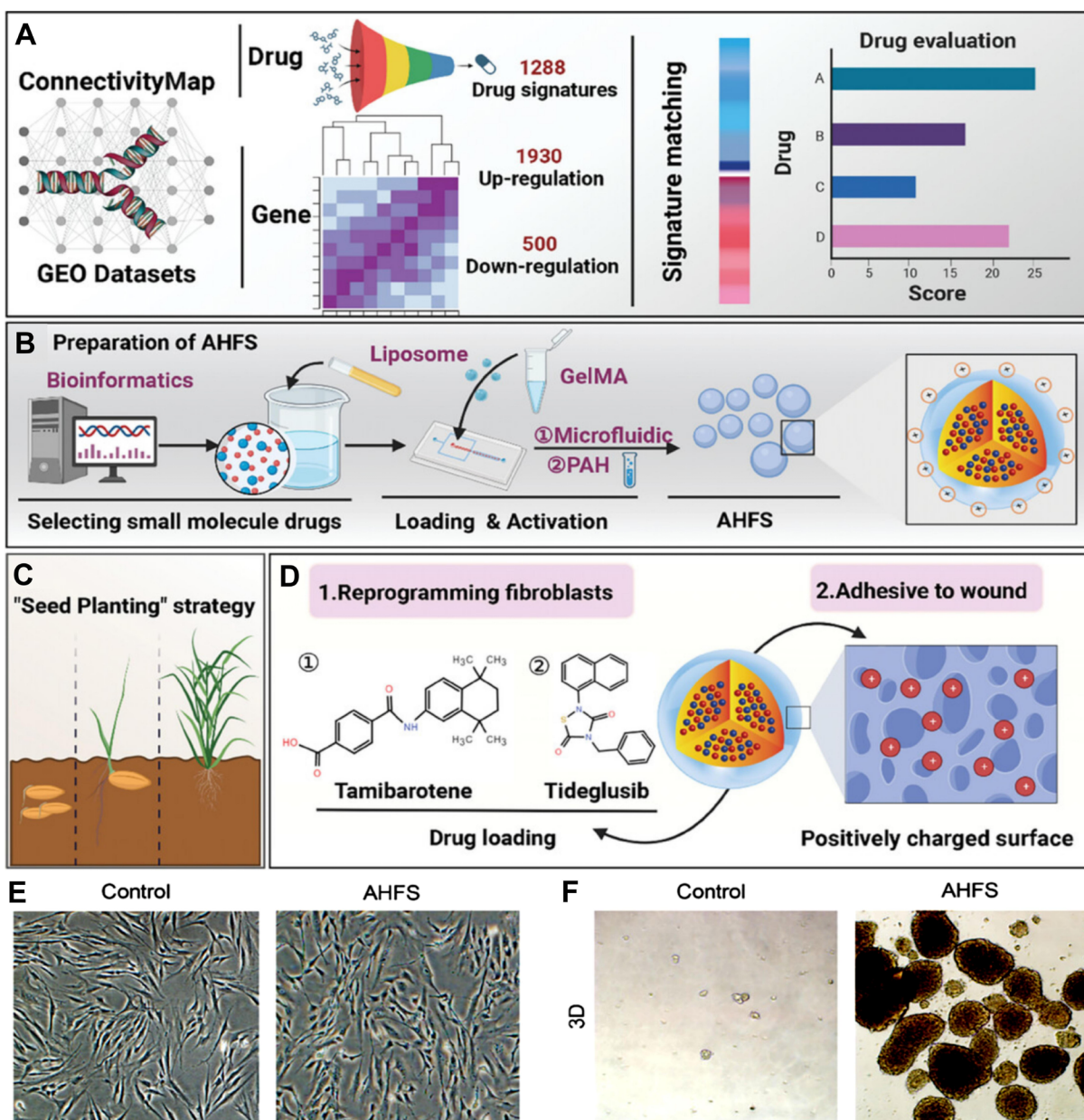
Three-dimensional bioprinting is recognized as an emerging technology that enables the precise deposition of materials and cells in a repeatable and high-throughput manner, thereby allowing the construction of complex architectures according to predefined patterns.<sup>51,52</sup> This technology is utilized for the accurate reconstruction of intricate structures, such as rete ridges, thereby enhancing basement membrane formation and promoting epidermal proliferation and differentiation.<sup>53,54</sup>

Leveraging the temperature-dependent shear-thinning properties of GelMA, it is extensively employed as a

foundational bioink component. Through multi-nozzle printing techniques, DPCs and keratinocytes are precisely positioned at specific geometric coordinates within 3D space, effectively mimicking the dermal-epithelial junctional structures of natural follicles.<sup>36,55,56</sup> In a study by Catarino *et al.*,<sup>10</sup> spheroids were precisely printed within a pre-gelled dermal layer containing fibroblasts by co-printing DPCs and human umbilical vein endothelial cells. Supported by the migration of keratinocytes and melanocytes, the resulting tissues matured into follicle-like structures with a morphology and composition highly analogous to native cutaneous tissue. Notably, it has been observed that although cell viability may remain high, the printing process may trigger an inflammatory response, potentially compromising tissue functionality and the subsequent utility of 3D-printed models.<sup>57</sup>

The precise arrangement of cells, aligned with the anatomical structure of natural hair follicles, is achieved with micron-level positional accuracy. Furthermore,





**Figure 4.** Construction of active hair follicle seeds *via* microfluidics. (A) Bioinformatics screening. (B) Preparation of AHFSs. (C) "Seed planting" strategy. (D) Mechanism and drug loading. (E,F) *In vitro* evaluation. Scale bars: 200  $\mu$ m, 100  $\mu$ m; magnifications: 100 $\times$ , 40 $\times$ .

Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional; AHFS: Active hair follicle seeds; GelMA: Gelatin methacryloyl; GEO: Gene expression omnibus; PAH: Poly(allylamine hydrochloride). Reprinted with modifications from Ref.<sup>50</sup>

by adjusting the cross-linking density of the bioink, the synchronization of scaffold degradation kinetics with the maturation cycle of the HFOs is enabled, facilitating dynamic spatiotemporal matching. This rigorous spatial control directly induces EMI, significantly enhancing the hair-induction rate and structural uniformity.

#### 4.2. Microfluidic technology

Through micron-scale fluid manipulation, microfluidic

technology is recognized as an ideal platform for the large-scale fabrication of homogeneous droplets, thereby providing a dynamic culture environment for organoids. In a study by Huang *et al.*,<sup>58</sup> murine MSCs and epidermal progenitor cells (EPCs) were encapsulated within a GelMA core and a photocured catechol-grafted HA shell, resulting in the fabrication of GelMA-MSC/HA-EPC (G/HA) microspheres. Findings indicated that these G/HA microspheres exhibit ultrafast gelation, aqueous phase



**Table 2. Summary of advanced manufacturing technologies for hair follicle organoids**

Fabrication method	Matrices	Cell types	Size	Key features	Reference
3D bioprinting	GelMA/HAMA	HaCaT cells	110 $\mu\text{m}$	Excellent viscoelastic and physicochemical properties, 3D printability, cytocompatibility, and functionality	Kang <i>et al.</i> <sup>26</sup>
3D bioprinting	Human collagen	DPCs, HUVECs, FBs	100 to 250 $\mu\text{m}$	Morphology and composition grossly mimicked native skin tissue	Catarino <i>et al.</i> <sup>10</sup>
3D bioprinting	Gelatin/alginate	FBs, HUVECs, DPCs, EPCs	250 $\mu\text{m}$	Controllable formation of self-aggregating spheroids of DPCs in a physiologically relevant ECM and the initiation of EMI	Kang <i>et al.</i> <sup>36</sup>
3D bioprinting	PCL, collagen	–	142.4 nm	Hair follicle-penetrating nanoparticles, a promising avenue for targeted antibiotic delivery	Aliyazdi <i>et al.</i> <sup>56</sup>
3D bioprinting/micro/nano fabrication	Type I collagen	DPCs, FBs, KCs	500/700 $\mu\text{m}$	HF-like microwells in 3D-reconstructed dermis, where genetically/extrinsically reprogrammed cells could be arranged easily into a physiologically relevant conformation	Abaci <i>et al.</i> <sup>55</sup>
Microfluidic	GelMA/HA	MSCs, EPCs	100 to 250 $\mu\text{m}$	Ultrafast gelation, aqueous phase separation, excellent biocompatibility, and superior wet adhesion	Huang <i>et al.</i> <sup>58</sup>
Micro/nano fabrication	PDMS	MSCs, EPCs	200 to 300 $\mu\text{m}$	Manipulating cell adhesivity with Y27632 triggers a transition from dumbbell-shaped to core-shell-structured aggregates	Kageyama <i>et al.</i> <sup>62</sup>

Abbreviations: 3D: Three-dimensional; DPCs: Dermal papilla cells; ECM: Extracellular matrix; EMI: Epithelial–mesenchymal interaction; EPCs: Epidermal progenitor cells; FBs: Fibroblasts; GelMA: Gelatin methacryloyl; HA: Hyaluronic acid; HaCaT: Human adult low calcium high temperature cells; HAMA: Hyaluronic acid methacryloyl; HF: Hair follicle; HUVECs: Human umbilical vein endothelial cells; KCs: Keratinocytes; MSCs: Mesenchymal stem cells; PCL: Polycaprolactone; PDMS: Polydimethylsiloxane; Y27632: Rho-associated protein kinase inhibitor Y-27632.

separation, excellent biocompatibility, and superior wet adhesion. Furthermore, cell proliferation was promoted, and the sustained release of growth factors was maintained by the G/HA microspheres. Upon transplantation into the back dermis of nude mice, efficient hair follicle generation was achieved (Figure 5). This simplified method for preparing bilayered cellular spheres is anticipated to advance current medical technologies for hair regeneration.

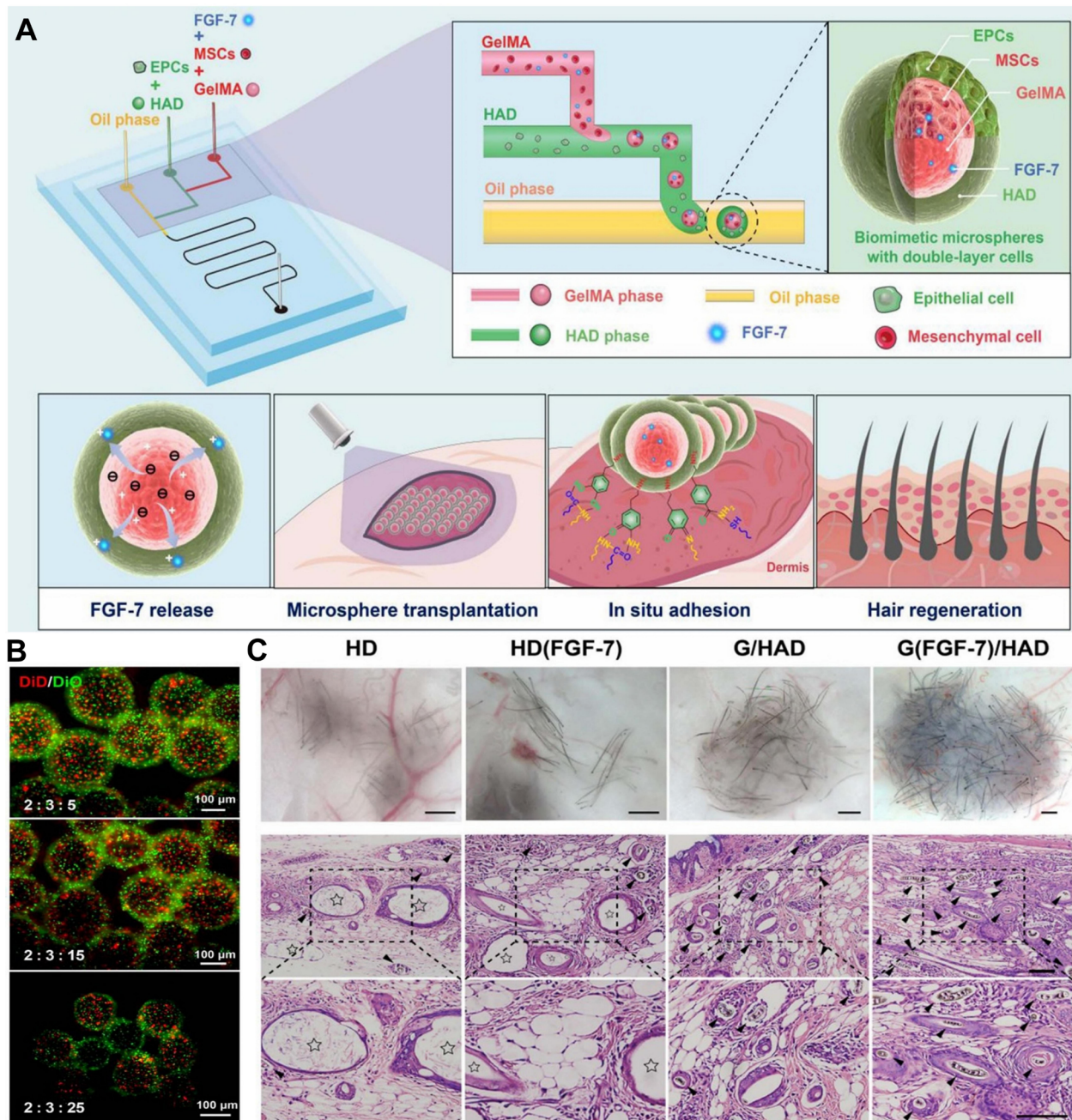
Polarization of the hair follicle is found to be influenced by fluid shear stress through mechanotransduction signals, such as those mediated by the Yes-associated protein/transcriptional co-activator with PDZ-binding motif pathway.<sup>59,60</sup> Within microfluidic chips, the flow and *in situ* solidification of GelMA solutions can be guided through the design of microchannels. For instance, mechanical constraints generated by micropillar arrays have been utilized to simulate shear-stress environments; such mechanical stimulation has been observed to promote the compaction of DPC aggregates, thereby enhancing their capacity to induce the formation of the hair shaft in epithelial cells.<sup>55</sup> In addition to the simulation of cutaneous

interstitial flow, the regulation of HFO maturation rates may be achieved in the future by establishing stable growth factor concentration gradients.

### 4.3. Micro/nano fabrication technology

To meet the requirements for preclinical drug screening, HFOs must be scalable and standardized. Microwell arrays are utilized, in which thousands of micrometer-scale indentations are fabricated on GelMA surfaces via physical templates. By seeding cells into these microwells, the formation of uniform cellular aggregates is induced, thereby eliminating individual variability.

The development of GelMA microneedles using polydimethylsiloxane (PDMS) molds is identified as a highly promising approach.<sup>61</sup> A notable practical application was designed by Kageyama *et al.*,<sup>62</sup> in which the tips of the microneedles were composed of GelMA-encapsulated organoids containing 5% GelMA hydrogel and cryoprotectants (CPAs) with 5% dimethyl sulfoxide. During fabrication, cells were compressed by centrifugal force, and the GelMA hydrogel functioned as the



**Figure 5.** Microfluidic fabrication of double-layered biomimetic microspheres. (A) Fabrication and application mechanism. (B) Microscope image of GelMA-encapsulated DiD-dyeing MSCs with outer HAD-encapsulated DiO-dyeing EPCs at different oil phase flow rates. Scale bars: 100  $\mu\text{m}$ ; magnification: 40 $\times$ . (C) *In vivo* regeneration evaluation. Scale bars: 200  $\mu\text{m}$ , 100  $\mu\text{m}$ , 100  $\mu\text{m}$ ; magnification: 40 $\times$ , 40 $\times$ , 100 $\times$ .

Abbreviations: DiD: 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine; DiO: 3,3'-dioctadecyloxycarbocyanine perchlorate; EPCs: Epidermal progenitor cells; FGF: Fibroblast growth factor; GelMA: Gelatin methacryloyl; HAD: Hyaluronic acid; HD: Hanging-drop. MSCs: Mesenchymal stem cells. Reprinted with permission from Ref.<sup>38</sup> Copyright © 2009 IOP Publishing Ltd.

ECM to mimic embryonic hair follicle structures. The photocrosslinked GelMA scaffold maintains a morphology conducive to EMI following the melting of ice crystals, while the CPAs provide protection against ice crystal-induced cellular damage. Furthermore, the large-scale preparation

(e.g., 5,000 microwells) of uniform organoids was achieved using oxygen-permeable PDMS microarray devices. This approach effectively prevents central cell death caused by hypoxia, ensuring the viability and functional consistency of the generated organoids.

## 5. Functionalization strategies and delivery applications

While advanced manufacturing technologies provide the necessary spatial constraints and structural framework for HFOs, maintaining their long-term functionality requires more than just physical architecture. The morphological maturation and periodic cycling of the hair follicle are heavily dependent on complex biochemical signaling inflection points. Therefore, leveraging the drug-loading capabilities of hydrogels to deliver these dynamic molecular cues is essential. By leveraging the drug-loading and sustained-release capabilities of GelMA hydrogels, biomimetic “biochemical signaling inflection points” can be provided for HFOs, thereby significantly enhancing their induction efficiency and functionalization levels.

### 5.1. Synergistic delivery of cells and bioactive factors

The successful development of organoids is highly dependent on intercellular interactions and the activation of specific signaling pathways, suggesting that the budding of HFOs requires support from high concentrations of growth factors. The activation of the Wnt/beta-catenin pathway is identified as the gold standard for hair follicle initiation. In a study by Ji *et al.*,<sup>50</sup> hydrogel microspheres were developed to activate the trichogenic potential of cells *in situ* through the synergistic delivery of fibroblasts and small-molecule inducing factors. Within this synergistic system, not only was physical support provided, but high-concentration signaling centers were also constructed at the micrometer scale, effectively simulating the placode formation process observed during embryonic development. Furthermore, the sequential release of factors was achieved through multi-layered scaffolds, enabling organoids to receive precise molecular instructions at distinct developmental stages, thereby significantly enhancing the regeneration efficiency of functional hair follicles.

### 5.2. Advanced functionalization of hair follicle organoids for complex wound environment

When transplanted into complex alopecic or severe wound environments, unmodified HFOs often face harsh conditions, such as excessive inflammation or androgenic inhibition. Therefore, to ensure successful engraftment and *in vivo* regeneration, GelMA hydrogels must be equipped with advanced functionalization strategies, such as extracellular vesicles (EVs) and gene-editing tools.

Extracellular vesicles are recognized as reliable intercellular messengers due to their capacity to transport proteins, lipids, and nucleic acids. The delivery of these molecular cargoes influences various physiological and pathological processes in both parental and recipient cells, and significant potential has been demonstrated in fields

such as wound repair, bone regeneration, and hair follicle regeneration.<sup>63-68</sup> It has been established that EVs secreted by DPCs can encapsulate and transmit signaling proteins, such as Wnt3a and Wnt7a, along with specific microRNAs (e.g., miR-218-5p). Through these mechanisms, quiescent HFSCs are activated and transitioned into the anagen phase.<sup>69-71</sup> Furthermore, milk-derived exosomes have been observed to accelerate the transition of the hair growth cycle from telogen to anagen, a process driven by the activation of the Wnt/ $\beta$ -catenin pathway.<sup>72</sup> Significant advantages are associated with EV-based strategies compared to direct cell seeding for organoid construction. EVs exhibit excellent biocompatibility, thereby mitigating the risks typically associated with allografting. Additionally, the concentration, storage, and standardized quality control of EVs are more readily facilitated, providing a highly feasible paradigm for the large-scale clinical application of hair follicle regeneration.

The remodeling of organoids via gene regulation is facilitated to target specific alopecic mechanisms, such as androgenetic alopecia. The precise knockdown of inhibitory genes in mesenchymal cells is achieved by encapsulating small interfering RNA or microRNA targeting Dickkopf-related protein 1 in GelMA.<sup>73-75</sup> This molecular-level remodeling has been shown to lift the microenvironmental blockade on Wnt signaling, thereby allowing organoids to retain active trichogenic inductivity, even within adverse androgenic environments. Nevertheless, since nucleic acid molecules are highly susceptible to degradation by environmental nucleases and face significant barriers to crossing cell membranes, the development of highly efficient delivery systems is deemed essential. In conjunction with nanocarriers, the stabilization of nucleic acid drugs within the GelMA matrix is anticipated to enable sustained cell transfection within organoids and provide a feasible pathway for developing intelligent hair follicle regeneration systems.

### 5.3. Application of small-molecule drugs in alopecia model screening

Pharmacological intervention is recognized as the primary modality for alopecia management, with the initial phase of drug discovery typically involving screening compounds in traditional two-dimensional (2D) cell cultures. However, to ensure accurate drug screening, the adoption of cell culture methodologies that more closely recapitulate the *in vivo* microenvironment is necessitated. Furthermore, the prioritization of terminal morphological transitions—such as hair follicle induction and growth—over transient cellular responses observed in 2D cultures (e.g., cell proliferation and gene expression) is identified as a critical metric. Using HFOs, candidate drugs, such as Janus kinase inhibitors, can be loaded at various



concentrations, enabling rapid identification of potential therapeutic agents for hair regeneration. This paradigm is considered more efficient and ethically compliant than conventional animal experimentation. For instance, the small-molecule compound cinnamic acid has been observed to significantly promote hair bud elongation by upregulating oxytocin receptor expression. In comparison to human hair follicle tissues—which are often limited in clinical accessibility—HFOs provide a more uniform, high-throughput platform and serve as indispensable tools for in-depth understanding of hair follicle morphogenesis and pharmacological evaluation.<sup>76,77</sup>

## 6. Conclusion

Current *in vitro* HFO models predominantly rely on passive diffusion for nutrient supply, which limits organoid size and results in central necrosis during long-term culture. A functional circulatory system is indispensable not only for survival but also for the effective integration of the graft into the host vascular network post-transplantation. Moreover, the hair follicle is a highly innervated organ, and nerve-derived signals are crucial for stem cell activation and cycling. Future strategies should prioritize the synergistic construction of neuro-vascular networks within GelMA systems. Utilizing advanced multi-material bioprinting and microfluidic technologies to co-pattern endothelial cells and neural progenitors alongside HFOs will be essential for mimicking the complex *in vivo* neurovascular cross-talk.

The transition from laboratory-scale fabrication to commercially viable production is hindered by the high cost of cell expansion and the manual nature of current organoid assembly protocols. This reliance on manual intervention inevitably leads to substantial batch-to-batch variability in organoid morphology and inductive capacity. To achieve clinical scalability, it is imperative to establish automated, industrial-grade production lines. The integration of robot-assisted bioprinting with artificial intelligence-driven quality control—employing machine learning algorithms to monitor organoid growth kinetics in real time—will be essential to ensure the standardization, reproducibility, and high-throughput production of clinical-grade HFOs.

As HFO technology progresses toward human applications, it intersects with complex ethical and regulatory challenges. Concerns regarding the sourcing of stem cells, the safety of gene-editing interventions, and the long-term stability of engineered tissues must be rigorously addressed. Future efforts must prioritize developing Good Manufacturing Practice-grade synthesis protocols and standardizing cross-linking parameters to align with Food and Drug Administration safety frameworks for regenerative medicine. Furthermore, creating comprehensive legal frameworks that define the safety standards for “living” therapeutics will be the essential

pathway for the safe clinical deployment of this technology, ultimately offering a transformative solution for patients suffering from alopecia and severe skin injuries.

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## Conflict of interest

The authors declare they have no competing interests.

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## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

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Not applicable.

## Further disclosure

Figures 1 and 2 were created with BioRender (agreement numbers: ZQ29AYC2X4, RH29AYBX9O).

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