Therapeutic Delivery

Engineering the future of hair follicle regeneration and delivery

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"The use of these reliable *in vitro* HF organoid models would allow the study of drug efficacy and uptake, hence, mimicking *in vivo* conditions for developing possible strategies for anti-AGA medications. Prospective models would also gravitate away from mice models to verify the inductivity of bioengineered spheroids."

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Hair loss can have an adverse impact on one's self-esteem and interpersonal relationships. Alopecia, a clinical term used to describe hair loss, affects countless individuals worldwide. Androgenetic alopecia (AGA), the most predominant form of alopecia, manifests itself in approximately 70% of alopecia cases [1]. The US FDA has approved minoxidil and finasteride for AGA. However, minoxidil requires long periods of use and relapses upon discontinuation is common [1]. Also, finasteride use is related to metabolic syndrome and only indicated for males [2]. Extensive studies have been conducted with traditional and herbal medicines to derive bioactives against AGA [3–5]; however, the lack of clinical trials has diminished its translational potential for public administration. As an alternative to medications, clinicians have long resorted to transplanting hair follicles (HFs) either from the patients' own peripheral hair-bearing regions or from donor skin, to balding regions [6]. However, grafts are susceptible to mechanical injury, dehydration, chemical solutions used for storage and hypoxia [7]. These factors, combined with a dearth of donor HF, diminish the quality and quantity of viable HFs available for transplantation. Therefore, in this article, we explore existing tissue engineering technologies for *de novo* HF regeneration and delivery, which could potentially be used as *in vitro* organoid models to study the effects of future drug delivery.

HF regeneration technologies

In the recent decade, researchers have been investigating novel approaches to regenerate new HFs. Garza *et al.* have reported a defect in the conversion of HF stem cells to CD200 and CD34-positive progenitor cells in the pathogenesis of AGA albeit the presence of HF stem cells within the balding scalp [8]. As such, groups have been exploring methods to convert regular stem cells into progenitor cells to re-activate hair growth. Follica (www.follicabio.com) explored the possibility of skin disruption on the balding scalp to recreate an 'embryonic window' for epithelial stem cells, which reside in the scalp, to differentiate toward a hair follicular fate. Replicel (https://replicel.com/) explored the transfer of healthy outer root sheath cells to deficit sites on the balding scalp to rejuvenate new HFs. Intercytex and Aderans Research Institute explored the possibility of hair follicular cell expansion *in vitro*, aiming to produce a reserve of HF for implantation [6]. Healthy HF cells are removed from patients and multiplied *in vitro* before being injecting back into the bald regions.

Tissue engineering: the future of HF regeneration & delivery

Higgins *et al.* highlighted changes to transcriptome profile between freshly isolated native human dermal papilla (DP) and DP cells cultured in monolayers for several passages, with many transcripts involved in HF development [9]. These expressions were partially restored when they attempted to culture DP cells in hanging drop cultures to create a microenvironment favoring self-aggregation. However, the inability to regulate the shape and size of each aggregate

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and the lack of scalability has limited its practical usefulness [10,11]. Efforts have thus been made to optimize and recapitulate the 3D microenvironments favorable for culturing hair follicular cells *in vitro*. Tissue engineering aims to regenerate biological tissues and organs by using cells within an artificially created supporting scaffold [12,13]. In the light of HF regeneration, it is a potential approach to reconstruct HFs from dissociated cells and recreate 3-D microenvironments with *in vivo*-like conditions. The following describes the strategies employed in the current state and development of HF regeneration and delivery.

Restoring epithelial influences on DP

When DP cells are removed from their HF microenvironment and cultured, they immediately lose contextual and positional cues from the surrounding epithelial cells [14]. However, this property can be restored if appropriate epithelial influences are provided. Previous studies have demonstrated that co-culture with keratinocytes maintains the *in vivo* characteristics of the DP [15,16]. Qiao *et al.* have shown that *in vitro* aggregated mouse follicular cells (DP cells and keratinocytes) developed partially formed hair structures while in culture within methylcellulose-coated wells [17]. Upon implantation into nude mice via incision, the protohair became mature hair and persisted for at least 6 months. Havlickova *et al.* reported the use of a mixture of rat collagen I and Matrigel Basement Membrane Matrix for the preparation of human folliculoid microspheres consisting of outer root sheath keratinocytes and DP cells [18]. This model has allowed for close interaction between the outer root sheath keratinocytes and DP cells, exhibits HF-type keratinization (*K6*) and displayed expression of versican, a chondroitin sulfate proteoglycan essential in HF formation. Lee *et al.* mixed epidermal and dermal cells from new-born mice, cultured them on Integra™ matrix (porous matrix of cross-linked bovine tendon collagen and glycosaminoglycan and a silicone layer) [19]. 8 days after grafting on the back of athymic mice, hair germ started to appear and developed into hair pegs, and 4 days later complete HFs were observed.

Restoring positional relationships of hair follicular cell composites

Previous *in vivo* HF reconstitution assays have demonstrated that co-grafted dissociated epithelial and dermal cells re-position themselves spontaneously to imitate their anatomical relationship *in vivo*, suggesting the importance of the positional relationship between epithelial and mesenchymal cells in HFs toward follicular morphogenesis and epithelial–mesenchymal interactions [20]. Thus, artificially assembling cell composites mimicking the HF bulb structure instead of using dissociated cells has been proposed to enhance epithelial–mesenchymal interactions. Pan *et al.* fabricated microstructured polyethylene glycol diacrylate hydrogels resembling the physiological architecture of HF, in which the dermal cells were encapsulated within the gel compartment, separate from the epidermal cell populations [21]. Similarly, Lim *et al.* developed a method to assemble DP and normal human epidermal keratinocytes in close proximity within 3D fibrous hydrogel scaffolds using two oppositely charged polyelectrolyte solutions [22]. Structures akin to native hair bulb arrangement were observed when these cell–fiber constructs were implanted subcutaneously into severe combined immunodeficiency mice. Researchers have also demonstrated the use of suitable biomaterial surfaces to initiate spontaneous assembly of homotypic cells into spheroidal microtissues without the aid of surface microfabrication [23–25]. Yen *et al.* exploited the differential adherence of keratinocytes and DP cells on poly(ethylene-co-vinyl alcohol) to form hybrid cell spheroids that have a preferential compartmented core-shell structure, in which aggregated DP cell core was surrounded by a keratinocyte shell [26].

Delivery of follicular cells for hair reconstitution *in vivo*

Hair reconstitution assays in mice are beneficial for learning hair-inductive properties of isolated cell populations. One of the earliest models used was the chamber assay, developed by Lichti *et al.*, in which dermal and epithelial cells were seeded inside a chamber consisting of a cylinder inserted through a full-thickness skin lesion in mouse dorsal skin, covered by a dome [27]. Usually the chamber was removed after a week and hair neogenesis can be observed from the back skin within 3 weeks by using cells from mouse neonates [28]. The second method, known as the patch assay, involved injecting dissociated murine neonatal epidermal and dermal cells hypodermically into the back skin of immunodeficient mice [20]. The injected epidermal cells aggregated into spheres, which were observed as nodules in the host skin, while dermal condensates outside the spheres induced hair follicular growth [29]. The third method, known as the flap assay, was developed by Qiao *et al.* and it involved first producing a skin flap in the dorsal skin of nude mice [30]. This assay used both the dermal cells and the embryonic mouse epidermis, which provided the keratinocyte component of the induced follicles. The grafts were placed under the skin flap in the host athymic mouse where the skin graft will develop hair if the dermal cells were hair-inductive.

Conclusion & future perspective

The tightly regulated molecular and cellular events involved in HF development increase the complexity of designing functional human HFs *de novo* for transplantation. The absence of essential skin features such as vasculature, sebaceous glands and innervation may attribute to currently bioengineered HFs having incomplete structures. Future models would require greater exploration into growing HF *in situ*, within suitable 3-D microenvironments containing these essential skin features. Tissue engineering enables compartmentalization of multi-organoid systems supplying all these essential components necessary for regenerating HFs. Other systems such as microfluidics and perfusion models might be relevant to ensure model sustainability. The use of these reliable *in vitro* HF organoid models would allow the study of drug efficacy and uptake, hence, mimicking *in vivo* conditions for developing possible strategies for anti-AGA medications. Prospective models would also gravitate away from mice models to verify the inductivity of bioengineered spheroids. The use of immunodeficient host mice for *in vivo* models may have limited translatable research outcomes to humans due with the absence of host immunity in the nude mice, for instance. Greater emphasis would also be placed on more rapid and mass HF transplantation technologies, while in amalgamation with high-throughput systems for supplying an endless reserve of readily available HFs.

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