Cell-based treatments for hair loss: research update on "hair cloning"

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The hope of cultured follicular cell implantation, or hair cloning, has held a special place in the imagination of hair restoration surgeons and the public alike for many years. Several decades of basic animal research established the seeming inevitability that cell therapy would be a successful treatment for alopecia by this point in time, with the attendant hope for unlimited hair. Yet, the current situation suggests that cell-based treatments are no closer to successful realization than when the author last reviewed this topic 10 years ago.[1] The present review will cover published research over the past 10 years. The basic rationale and conceptual framework for using cell therapy to treat hair loss has been discussed in prior reviews and will not be covered here.[2,3]

Although clinical trials are underway using cultured follicular cells, no published results in humans were available at the time of this writing. Furthermore, because of the inherent commercial potential of research findings, it can be assumed that much more is known than is publicly available. While knowledge of the cellular and molecular mechanisms underpinning hair growth has progressed substantially over the past 10 years, it appears that this has not yet translated into a clinically useful method to treat hair loss in humans.

The basis for cell therapy began in the fundamental research of normal mammalian hair growth. It had been established that hair growth occurred because of the dynamic interaction between epidermal and mesenchymal cell populations within the hair bulb. Using the rat whisker as a model, Oliver showed that the spherical aggregate of mesenchymal cells in the bulb known as the dermal papilla could induce new follicles when removed from the whisker and subsequently implanted into skin that normally lacks follicles.[4] Carrying this research further, Jahoda showed that rat whisker dermal papilla that had been cultured *in vitro* could induce new hair growth when implanted into incisions in the rat ear.[5] This research, published in the mid-1980s, initiated the race to develop cell-based treatments for hair loss. In the ensuing years, several commercial ventures were launched based on the work of Oliver and Jahoda.

Over the past 10 years, we have seen a major commercial venture fail that was based on using implanted cultured autologous DP in humans. While some increased hair growth was claimed, it was largely agreed that simply implanting cultured DP in humans did not result in clinically meaningful hair induction. A second commercial venture combining cultured DP and keratinocytes is also being put through human clinical trials at the current time.[6] While the results have not been published, early reports are that no follicular neogenesis has been observed, although some thickening of native hair may result, which may be clinically beneficial. What has become increasingly clear is that methods that routinely induce new hair follicles in mice and rats are largely unsuccessful in humans.

A third venture is based on using not cultured dermal papilla, but the adjacent mesenchymal dermal sheath "cup" cells (DSC).[7] This is based on the research of McElwee who showed that implantation of cultured DSC cells from mouse whiskers could be injected into the ear, resulting in "colonization" of the dermal papilla of ear hair follicles with whisker cells programmed to produce thicker hair.[8] Early reports from clinical trials using cultured DSC in humans suggest it is safe and may produce some hair thickening.

It is important to note here that no published reports exist of reproducible de novo hair follicle induction in humans (i.e., the formation of entirely new hair follicles in alopecic skin). What the current commercial ventures mentioned above do appear to show is that implantation of cultured follicular cells may result in clinically apparent thickening of native hair follicles. Whether these implanted cells incorporate into the structure of the native follicle and survive cycling or whether they merely secrete stimulatory agents that transiently promote thicker hair is an important distinction. If it is the former, then such a treatment may represent a true breakthrough in alopecia treatment with long-lasting or permanent effects. If it is the latter, then the expense of cell-based treatment may not justify the temporary benefits. Furthermore, the increasing popularity of injecting platelet rich plasma (PRP) for hair loss,[9,10] as well as an injectable treatment under development containing growth factors secreted by cultured neonatal fibroblasts (Histogen),[11] may produce similar gains at a fraction of the expense, without the burdensome regulatory requirements of cell therapy.

Animal Research and Study Models

These commercial ventures have their origin in basic research conducted on rat or mice. The seminal studies by Oliver and Jahoda were based on manipulating dissected rat vibrissa (whisker), which later proved cumbersome and limiting as research methods became more advanced. Over the years, more refined models have been developed that have allowed researchers to study the intricate inner workings of follicular neogenesis.

For many years, the silicon chamber model, pioneered by Ulrich and Yuspa, was the dominant model used by researchers in this area.[12] Dermal and epidermal cells, either fresh or cultured, were combined in full-thickness wounds on the backs of immunodeficient mice and covered with a bell-shaped silicon chamber that confined and protected the cells; after one week, the chamber was removed, and new hair growth was apparent within three weeks.

Qiao created a "flap graft" model that dispensed with the need for the chamber, and allowed implantation of trichogenic dermal and epidermal cells below a flap of host skin.[13] This model still required the implantation of, and subsequent removal of, a silicone sheet. Stenn developed a "Patch Assay" that consisted of injecting dermal and epidermal cells subcutaneously into immunodeficient mice. The resulting "hair ball" consists of hair bulbs located centrally with hair growth occurring radially outwards.[14]

Almost all *in vitro* models have relied on mouse, usually embryonic, as the source of cells. It has been noted by researchers that successful hair follicle induction using mouse or rat cannot necessarily be translated into success using human cells.

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Although Stenn did report success with the "Patch Assay" using adult human dermal cells, it is important to note that the epidermal cells were from human fetus and the host was immunodeficient mouse.[15]

One report stands alone in using only adult human cells and tissue. Krugluger described a human skin organ model in which injections of human DP and epithelial cells resulted in follicle induction and growth of vellus type hair.[16] However, no follow-up studies from this group, or using this technique, have been reported in the many years since its original publication.

Cell Culturing Conditions

Early experiments clearly showed that culturing conditions determined the success of dermal papilla–induced follicle morphogenesis. Cultured dermal papilla gradually lose their inductive ability as they are passaged and expanded in culture. Yoshizato showed that the presence of keratinocytes or keratinocyte-conditioned media could keep cultured DP inductive through many passages.[17] Subsequent to this, it was found that the factors present in conditioned media were soluble Wnt proteins excreted by keratinocytes whose primary function appears to be keeping beta-catenin in the DP active, which is essential for maintaining inductive potential.[18,19]

In addition to the presence of soluble Wnt factors to keep beta-catenin in cultured DP active, it has been found that three-dimensional culture conditions favor subsequent follicle induction over standard two-dimensional techniques.[20] Several reports have shown that cultured DP cells are more inductive when coaxed to aggregate into spheres rather than as the standard monolayers present in two-dimensional culture.[21-23]

Cell Implantation Technique

When the first reports of successful follicle induction in animals using cultured cells was reported in the mid-1980s, it was assumed that not only would success in humans follow shortly, but that the envisioned treatment would consist of injections of dissociated cells directly into the scalp, where they would induce the formation of new follicles. Over the past 10 years, it has become increasingly clear that injection of dissociated dermal cells, with or without epidermal cells, is largely unsuccessful in inducing new follicle formation in humans.

Many reports using animal models have appeared focusing on modifying and augmenting the method of culture and implantation. The rationale is that by optimizing the culture and delivery of cells, follicle induction will be successful in humans. One report describes the comparative success of different implantation techniques on hair regeneration when using cultured DP alone (no epidermal cells). Cultured rat DP placed directly beneath the host epidermis using the "hemi-vascular sandwich" technique showed superior hair induction.[24] These researchers claimed that this technique allowed use of dissociated DP cells alone, and that success was due to contact between DP cells and host epidermal cells as well as better oxygenation of the implanted cells.

Toward a New Paradigm

Current research appears to be leading toward a treatment paradigm where dermal and epidermal cells are combined for a period of time before implantation. In a method reported by Qiao, mouse dermal and epidermal cells were cocultured prior to implantation, resulting in the formation of primitive "proto-hairs" that could be subsequently transplanted, resulting in growth of mature, cycling hair.[25] It bears repeating that this involved animal cells, not human.

Along these lines, Lindner described the creation of "neo-papilla" using cultured human DP and components of extracellular matrix, followed by coculture with human keratinocytes and melanocytes. This resulted in the formation of *in vitro* follicles with vellus-like hair shaft growth.[26]

Similarly, Tsuji and colleagues reported that cultured DP and epidermal cells could be combined in a collagen gel to create a "bioengineered follicle germ," which could then be transplanted along with a fine suture that served as a guide for follicle directionality and connection to the outside epidermis, thus preventing cyst formation.[27] When transplanted into the hairless skin of mice, these bioengineered "hair germ" grafts took root and produced hair, and analysis of these hair follicles showed all correct layers of normal follicles and accessory structures including connection with surrounding host tissue (i.e., arrector pili muscles and nerve fibers).[28] Normal hair cycling occurred, confirming presence of necessary stem cell populations within the new hair follicles.

Furthermore, these researchers reported similar success using adult human follicle stem cells (DP and bulge-region derived epithelial cells), which may represent an important breakthrough. In a variation of their method, they implanted the bioengineered hair germ into the subrenal capsule of mice, providing a protected, vascularized space for induction to occur. After two weeks, mature hair follicles were seen in clusters and could be harvested for subsequent transplantation as 1- and 2-hair follicular units.[29] They suggested that these bioengineered hair grafts could be used in a manner similar to follicular unit transplantation (FUT). One might envision a treatment where the patient's follicles are shipped to the lab, where the cells are dissected out and grown first in organ culture, then matured in surrogate mice, before being harvested and shipped back to the surgeon where they could be implanted via the FUT technique.

Conclusion

Follicular cell implantation for hair loss remains an exciting possibility, but true hair multiplication is a long way off from practical reality in the clinic. Cell-based treatments may come online first as "hair thickening" treatments, where periodic scalp injections produce modest clinical gains similar to finasteride and minoxidil. True hair follicle neogenesis in humans has proven far more complex than that which has been routinely carried out in rats and mice, but intricate models are now being developed that keep alive the dream of unlimited hair.

References

1. Cooley, J. Follicular cell implantation: an update on "hair follicle cloning." *Facial Plast Surg Clin N Am.* 2004; 12:219-224.
2. Teumer, J., and J. Cooley. Follicular cell implantation: an emerging cell therapy for hair loss. *Sem Plast Surg.* 2005; 19(2):189-196.
3. Stenn, K., et al. Bioengineering the hair follicle. *Organogenesis.* 2007; 3(1):6-13.
4. Oliver, R.F. The experimental induction of whisker growth in the hooded rat by implantation of dermal papillae. *J Embryol Exp Morphol.* 1967; 18:43-51.

5. Jahoda, C.A., K.A. Horne, and R.F. Oliver. Induction of hair growth by implantation of cultured dermal papilla cells. *Nature.* 1984; 311:560-562
6. http://www.aderansresearch.com/ari_clinicupdates.html
7. http://www.replicel.com/wp-content/uploads/2012/Phase-I-IIa-Interim-Results.pdf
8. McElwee, K.J., et al. Cultured peri-bulbar dermal sheath cells can induce hair follicle development and contribute to the dermal sheath and dermal papilla. *J Invest Dermatol.* 2003; 121:1267-1275.
9. Greco, J., and R.J. Brandt. Preliminary experience and extended applications for the use of autologous platelet rich plasma in hair transplantation surgery. *Hair Transplant Forum Int'l.* 2007; 17(4):131.
10. Park, K.Y., et al. Platelet rich plasma for treating male pattern baldness (letter). *Dermatol Surg.* 2012(Dec); 38(12):2042-2044.
11. Zimber, M.P., et al. Hair regrowth following a Wnt- and follistatin-containing treatment: safety and efficacy in a first-in-man phase 1 clinical trial. *J Drugs Dermatol.* 2011; 10(11):1308-1312.
12. Weinberg, W.C., et al. Reconstitution of hair follicle development *in vivo:* determination of follicle formation, hair growth and hair quality by dermal cells. *J Invest Dermatol.* 1993; 100:229 236.
13. Qiao, J., E. Philips, and J. Teumer. A graft model for hair development. *Exp Dermatol.* 2008; 17:512-518.
14. Zheng, Y., et al. Organogenesis from dissociated cells: generation of mature cycling hair follicles from skin-derived cells. *J Invest Dermatol.* 2005; 124:867-876.
15. Zheng, Y., et al. Mature hair follicles generated from dissociated cells: a universal mechanism of folliculoneogenesis. *Dev Dyn.* 2010; 239(10):2619-2626.
16. Krugluger, W., et al. Reorganization of hair follicles in human skin organ culture induced by cultured human follicle–derived cells. *Exp Dermatol.* 2005; 14:580 585.
17. Inamatsu, M., et al. Establishment of rat dermal papilla cell lines that sustain the potency to induce hair follicles from afollicular skin. *J Invest Dermatol.* 1998; 111(5):767-775.
18. Kishimoto, J., R.E. Burgeson, and B.A. Morgan. Wnt signaling maintains the hair-inducing activity of the dermal papilla. *Genes Dev.* 2000; 14:1181-1185.
19. Soma, T., et al. Hair-inducing ability of human dermal papilla cells cultured under Wnt/β-catenin signaling activation. *Exp Dermatol.* 2012; 21(4):307-309.
20. Higgins, C.A., et al. Modeling the hair follicle dermal papilla using spheroid cell cultures. *Exp Dermatol.* 2010; 19(6):546-548.
21. Osada, A., et al. Long-term culture of mouse vibrissal dermal papilla cells and de novo hair follicle induction. *Tissue Eng.* 2007; 13(5):975-982.
22. Young, T.H., et al. Self-assembly of dermal papilla cells into inductive spheroidal microtissues on poly(ethylene-co-vinyl alcohol) membranes for hair follicle regeneration. *Biomaterials.* 2008(Sep); 29(26):3521-3530.
23. Huang, Y.C., et al. Scalable production of controllable dermal papilla spheroids on PVA surfaces and the effects of spheroid size on hair follicle regeneration. *Biomaterials.* 2013; 34(2):442-451.
24. Aoi, N., et al. Clinically applicable transplantation procedure of dermal papilla cells for hair follicle regeneration. *Tissue Eng Regen Med.* 2012; 6(2):85-95.
25. Qiao, J., et al. Hair morphogenesis *in vitro:* formation of hair structures suitable for implantation. *Regen Med.* 2008; 3(5):683-692.
26. Lindner, G., et al. De novo formation and ultra-structural characterization of a fiber-producing human hair follicle equivalent *in vitro. J Biotechnol.* 2011; 152(3):108-112.
27. Nakao, K., et al. The development of a bioengineered organ germ method. *Nat Methods.* 2007; 4(3):227-230.
28. Toyoshima, K.E., et al. Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches. *Nat Commun.* 2012; 3:784.
29. Asakawa, K., et al. Hair organ regeneration via the bioengineered hair follicular unit transplantation. *Sci Rep.* 2012; 2:424.◆