Experimental Evidence in Hair Restoration Procedures

Plucked Hair Survival and Growth Rate

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ABSTRACT

Background: Limitations of hair restoration procedures are the amount of hairs available and the invasiveness of follicular harvesting. **Objective:** The aim of this study was to compare conventional human micrografts and plucked hair follicles in an *in vitro* model in order to test hair growth rates for experimentally assessing the soundness of plucked follicle use in hair transplantation procedures. **Methods and materials:** A total of 100 conventional one-hair micrografts (group A; control) and a total of 80 plucked hair follicles (group B; experimental) were obtained from 14 healthy male patients. The length of each graft was measured immediately following isolation and at the end of the 10-day culture period. The Kruskal-Wallis one-way analysis of variance by ranks test was used in order to statistically analyze the data obtained. **Results:** A statistically significant difference was found between the growth rate of micrografts in control (mean 10-day shaft growth rate = 0.30mm) and experimental (mean 10-day shaft growth rate = 0.36mm) groups. **Conclusion:** The obtained data shows a higher plucked hair follicle growth rate compared to one-hair micrografts, which leads us to believe that plucked micrografts could be a useful and less invasive adjunct in the field of hair transplantation surgery. (*J Clin Aesthet Dermatol.* 2016;9(3):39–41.)

Hair transplantation for correction of androgenetic alopecia is one common aesthetic plastic surgery procedure performed on male patients.[1,2] The principal purpose of hair harvesting has always been to obtain the maximum survival and growth rate possible. Micrografting is a method of hair transplantation involving randomly assorted groups of (1–2) hairs, without considering the natural configuration of their follicular units, which are selected under loupe, microscope, or naked eye examination.[3] This type of graft is prepared by isolating each follicle as a whole, while maintaining a significant amount of tissue around the entire length of the follicle. Careful patient selection is very important, especially for African Americans and patients of Mediterranean origin on account of the curvilinear direction of their hair shafts, which could make graft preparation more difficult.[4] Micrografting technique has been used both for hair restoration of the scalp and the face after burn injuries[5,6] and particularly for eyebrow reconstruction[7] due to the fine control obtainable with this technique and the natural-looking results. Plucked human hair consists in hair follicles which have been stripped from the donor area, without any dissection or local anesthesia. As demonstrated in the authors' previous studies,[8,9] rapid proliferating hair follicle capacity probably depends on the presence of human hair follicle multipotent stem cells in the bulge region of the outer root sheath. These previously characterized cells[10] show an expression of proliferation marker Ki-67 and stem cell marker CD-34 in the follicle bulge region; the authors observed that plucked hair follicles have the same multipotent cell population as adult and fetal scalp hair follicles.[9] Although some authors[11–13] have recently described the presence of stem cells in plucked hair follicles, this was, to the best of the authors' knowledge, the very first study specifically designed to assess the survival and growth rates of human plucked hair follicles, hypothesizing their possible use in hair restoration surgery. The aim of this study was to compare conventional human micrografts and human plucked hair follicles in an *in vitro* model, to experimentally assess the soundness of plucked hair follicle use in hair restoration surgery.

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Figure 1. Human plucked hair follicle (no stain was used; original magnification x20)

Figure 2. Histologic analysis of a survived follicle showing a normal histologic appearance after 10 days in culture (Azan trichromic; original magnification x50).

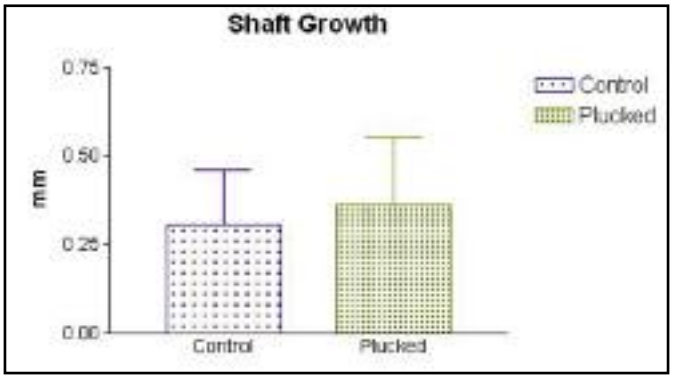

Figure 3. Statistically significant differences between the growth rate of control one-hair micrografts (mean 10-day growth rate = 0.30mm) and of experimental plucked hair follicles (mean 10-day growth-rate = 0.36mm).

MATERIALS AND METHODS

A total of 180 human hair follicles were obtained from 14 healthy male patients (age 28–42 years, mean 34 years) during routine excision of benign scalp lesions (e.g., naevi and cysts). The principles outlined in the Declaration of Helsinki were followed. Follicles were thus randomly assigned to one of the following groups: Group A (control; n = 100 follicles), conventional one-hair micrografts cultured as dissected; Group B (experimental; n = 80 follicles), plucked hair follicles (Figure 1). Isolation of anagen hair follicles was achieved by using a surgical blade, microscissors, and watchmaker's forceps under a stereo dissecting microscope (Axioskop MC100 – Zeiss, Oberkochen, Germany). Each follicle was isolated intact and as a whole while maintaining a significant amount of tissue around the entire length of the follicle. Hair grafts from both groups were cultured for 10 days,[14–17] and the hair follicles kept in 500µL of Williams' medium E (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) with supplements as follows: one percent fetal calf serum, 10µg/mL transferrin, 10µg/mL insulin, 10ng/mL sodium selenite, 10ng/mL hydrocortisone, 100 units/mL penicillin, 100µg/mL streptomycin, 2.5µg/mL fungizone. Supplemented medium was prepared fresh before experiments and changed every 72 hours. Follicles were kept free floating in individual wells of 24-well multiwell plates in a 37°C atmosphere, 5% CO_2, 95% air, and 100% humidity. This permitted detailed measurements to be made of the length of individual hair follicles. Follicles that lost normal follicular architecture owing to degeneration late in the culture period were not measured. The length of each follicle was measured at a magnification of 20x, immediately following isolation and at the end of the 10-day culture period, using a microscope (Wild M10 - Leica, Heerbrugg, Switzerland) with a calibrated eyepiece graticule; total follicle length was computed as the distance from the base of the bulb to the end of the shaft. Histology was carried out at the end of the 10-day culture period, by fixing the follicles in phosphate-buffered saline (pH 7.4) containing 10% paraformaldehyde, embedding them in paraffin wax, sectioning at 10µm thickness, and staining with the Heidenhein's "Azan trichromic" modified protocol.[18] The obtained data was statistically analyzed with the Kruskal-Wallis one way analysis of variance by ranks test.

RESULTS

Histologic analysis demonstrated that the survived follicles from both groups maintained a constant, normal histologic appearance (Figure 2), even after 10 days in culture. Furthermore, the morphology of most of the dissected follicles was retained for the entire 10-day period of culture. No statistically significant differences were found between the survival (considered as preservation of normal follicular architecture and absence of degenerative signs) rate of hair grafts from control group A and experimental group B. All grafts produced a measurable shaft elongation, and a statistically significant difference was found between the growth rate of control (conventional one-hair micrografts) grafts (mean 10-day growth rate = 0.30mm) and of experimental (plucked micrografts) grafts (mean 10-day growth-rate = 0.36mm) (Figure 3). The increase in length was always associated with a keratinized hair shaft.

DISCUSSION

The present study addressed the question of whether human adult plucked hairs are suitable for possible employment in hair restoration procedures. For this purpose, the authors assessed the survival and growth rate of human plucked hairs, as compared to that of conventional one-hair micrografts, using a dependable, reproducible, and quantifiable *in vitro* system. Indeed, hair-follicle culture is a reliable way of *in vitro* testing the effects of different procedures related to hair restoration surgery. Possible major advantages of transplanting plucked hairs could be a significant reduction in invasiveness and graft harvesting process time. Moreover, there are chances that hairs might re-grow at the donor site due to the presence of bulb stem cells retained in the sub-dermal layer. Finally, it is worth underlining that plucked hairs have demonstrated a statistically significant growth rate compared with single-hair micrografts. The authors believe that these stem cells may play a pivotal role in future hair transplantation procedures, proving that even plucked hair could be employed for this purpose. However, it should be taken into account that this was only an *in vitro* study, and that the obtained data should be assessed by means of further *in vivo* studies.

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REFERENCES

1. Unger WP. *Hair Transplantation.* 3rd ed. New York: Marcel Dekker; 1995.
2. Fan J, Raposio E, Nordström REA. Minigraft preparation in surgical hair replacement. *Scand J Plast Reconstr Surg Hand Surg.* 1997;31:83–86.
3. Rassman WR, Bernstein RM, McClellan R, et al. Follicular unit extraction: minimally invasive surgery for hair transplantation. *Dermatol Surg.* 2002;28:720–728.
4. Barrera A. Micrograft and minigraft megasession hair transplantation results after a single session. *Plast Reconstr Surg.* 1998;102:2237–2240.
5. Barrera A. The use of micrografts and minigrafts for the treatment of burn alopecia. *Plast Reconstr Surg*. 1999;103:581–584.
6. Motamed S, Davami B. Eyebrow reconstruction following burn injury. *Burns.* 2005;31:495–499.
7. Nordström RE. Eyebrow reconstruction by punch hair transplantation. *Plast Reconstr Surg.* 1977;60:74–76.
8. Raposio E, Filippi F, Levi G, et al. Follicular bisection in hair transplantation surgery: an *in vitro* model. *Plast Reconstr Surg.* 1998;102:221–226.
9. Raposio E, Guida C, Baldelli I, et al. Characterization of multipotent cells from human adult hair follicles. *Toxicol In Vitro.* 2007;21:320–323.
10. Lyle S, Christofidou-Solomidou M, Liu Y, et al. The C8/144B monoclonal antibody recognizes cytokeratin 15 and define the location of human hair follicle stem cells. *J Cell Sci.* 1998;111:3179–3188.
11. Gho CG, Braun JE, Tilli CM, et al. Human follicular stem cells: their presence in plucked hair and follicular cell culture. *Br J Dermatol.* 2004;150:860–868.
12. Webb A, Li A, Kaur P. Location and phenotype of human adult keratinocyte stem cells of the skin. *Differentiation*. 2004;72:387–395.
13. Aasen T, Izpisúa Belmonte JC. Isolation and cultivation of human keratinocytes from skin or plucked hair for the generation of induced pluripotent stem cells. *Nat Protoc.* 2010;5:371–382.
14. Raposio E, Cella A, Panarese P, et al. Power-boosting the grafts in hair transplantation surgery: evaluation of a new storage medium. *Dermatol Surg.* 1998;24:1342–1346.
15. Raposio E. *Clinical and Experimental Advances in Reconstructive and Aesthetic Surgery of the Scalp.* Tromsø, Norway: Tromsø University Press; 1998.
16. Raposio E, Cella A, Panarese P, et al. Effects of cooling micrografts in hair transplantation surgery. *Dermatol Surg.* 1999;25:705–707.
17. Raposio E, Caruana G. Scalp surgery: quantitative analysis of follicular unit growth. *Plast Reconstr Surg Glob Open.* 2015;3(10):e539. Published online 2015 Oct 20.
18. Heidenhein M. Uber die Maliorysche Bindegeweosfarbung mit Karmin und Azokarmin als Vorfarben. *Ztsch F Wissensch Wikr.* 1915;32:361–366.