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EXPERIMENTAL

Scalp Surgery: Quantitative Analysis of Follicular Unit Growth

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Background: Over the years, different kinds of hair transplantation have been compared in an attempt to overcome male pattern alopecia and, at the same time, maximize both the survival and growth rate of grafted hair. In this study, we have assessed the survival and growth rate of follicular units (FU) in an in vitro model, as compared with that of conventional hair micrografts, to experimentally evaluate and elaborate on the differences between these 2 approaches in hair transplantation procedures.
Methods: Group A (control; n = 100 follicles) was composed of hair micrografts, whereas FUs were assigned to Group B (experimental; n = 100 follicles, n = 35 FUs). Each group was cultured for a period of 10 days; the total stretch of follicles was measured soon after the harvest and 10 days later. The Kruskal-Wallis one-way analysis of variance on ranks test was used to perform statistical analysis.
Results: The growth rate of follicles from Group A (mean 10-day shaft growth rate = 0.30 mm) proved to be statistically different compared with that of Group B (mean 10-day shaft growth rate = 0.23 mm). Conversely, our data did not show any significant difference between the survival rate of hair grafts from these 2 groups.
Conclusions: Our data highlighted a reduced FU shaft growth compared with that of hair micrografts, corroborating, to a certain extent, the hypothesis that a significant amount of adipose tissue surrounding the follicle included in the graft may result in an inadequate nourishment supply to follicular cells. *(Plast Reconstr Surg Glob Open 2015;3:e539; doi: 10.1097/GOX.0000000000000524; Published online 20 October 2015.)*

Over the years, different kinds of hair transplantation have been compared in an attempt to overcome male pattern alopecia and, at the same time, maximize both survival and growth rates of grafted hair.

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Hair follicular transplantation, presented by Bernstein et al[1] in 1995, is grounded on hair emerging from the scalp in a set of 1–4 (5 in some occasions) terminal hair follicles. These follicles, together with 1 (rarely 2) vellus follicles, the associated sebaceous lobules, the insertion of the arrector pili muscles, its neural and vascular plexuses, and the adventitial collagen, represent the follicular units (FUs).[2] This whole structure must therefore be collected directly from the donor area during the harvesting procedure and then grafted into the recipient area.

Hair micrografts that were originally designed to improve the frontal hair line by making it look more

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natural[3,4] after hair transplantation also became useful in the grafting of eyebrow reconstruction[5] (ie, after-burn injuries). Furthermore, several surgeons have been performing the so-called "megasession," which consists in the transplantation of a large number of micrografts per session.[6–8] A micrograft is composed of 1–2 hairs, surrounded by minimal interfollicular tissue, not necessarily corresponding to the naturally occurring FUs.[2]

With this study, we compared the survival and growth rate of FUs and conventional hair micrografts, in an in vitro system, to experimentally evaluate and elaborate on the differences between these 2 approaches in hair transplantation procedures.

MATERIALS AND METHODS

We harvested 200 human hair follicles from 14 patients (sex: males; ethnic group: Caucasian; age: 28–42 years) during routine excision of benign scalp lesions (eg, nevi and cysts). All procedures were in accordance with the ethical standards of our institutional research committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Using a stereo dissecting microscope (Axioskop MC100—Zeiss, Oberkochen, Germany), anagen hair follicles were harvested by means of a surgical blade, microscissors, and watchmaker's forceps.[9–11] Two groups were realized with isolated follicles, which were assigned randomly to avoid possible selection biases. Group A (control; n = 100 follicles) was composed of conventional hair micrografts, which were cultured and isolated (Fig. 1), whereas FUs (Fig. 2) were assigned to Group B (experimental; n = 100 follicles, n = 35 FUs). A considerable quantity of tissue was maintained around each follicle. We had the use of 1-mm punches, by means of which we were able to detach FUs from the adjacent tissue down to the level of the subcutaneous adipose tissue.[12] Using a quantitative method previously described by Raposio et al,[9–11] hair grafts from both groups were cultured for 10 days, plunged into 500 µl of Williams medium E (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), which was changed every 72 hours. The following culture media were employed: 1% fetal calf serum, 10-µg/mL transferrin, 10-µg/mL insulin, 10-ng/mL sodium selenite, 10-ng/mL hydrocortisone, 100-units/mL penicillin, 100-µg/mL streptomycin, and 2.5-µg/mL fungizone. We kept all follicles at 37°C, 5% CO_2, 95% air, and 100% humidity, free floating in individual 24-well multiwell plate catch basins, our aim being to measure the stretch of each follicle[9–11] meticulously. After excluding those follicles with a degenerated architecture (the nonsurviving ones), the length of the remaining follicles was measured at a magnification of 20×, both immediately after isolation and at the end of the 10-day culture period. Measurements were performed through a microscope (Wild M10—Leica, Heerbrugg, Switzerland) with a calibrated eyepiece reticule; total follicle length was evaluated from the stretch between the base of the bulb to the tip of the shaft. At the end of the 10-day culture period, histological analysis was carried out by fixing the follicles in phosphate-buffered saline (pH 7.4) with 10% paraformaldehyde, implanting in paraffin wax, sectioning at 10-µm thickness, and staining with Heidenhein's "Azan trichromic" modified protocol.[13] The Kruskal-Wallis one-way analysis of variance on ranks test was used to statistically analyze the data obtained.

Fig. 1. Conventional one-hair micrograft.

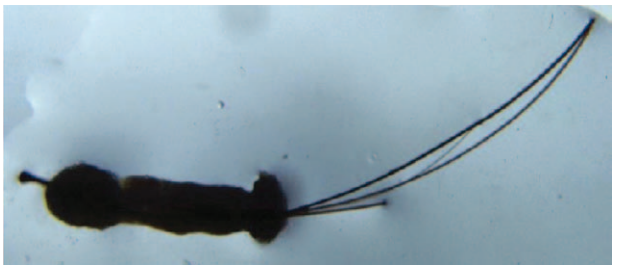

Fig. 2. Follicular unit.

RESULTS

The growth rate of follicles from Group A (hair micrografts—mean 10-day shaft growth rate = 0.30 mm) proved to be statistically different ($P < 0.05$) compared with that of Group B (FUs—mean 10-day shaft growth rate = 0.23 mm) (Fig. 3). Conversely, our data did not show any significant difference between the survival rate of hair grafts from these 2 groups (Group A: 85.7%, Group B: 88.6%).

Histological analysis revealed an average appearance for survived follicles from both Groups, even after 10 days in culture, proving that the measurable increase in length over 10 days (due to keratinized hair shaft production) was not related to any damage to hair follicle architecture.

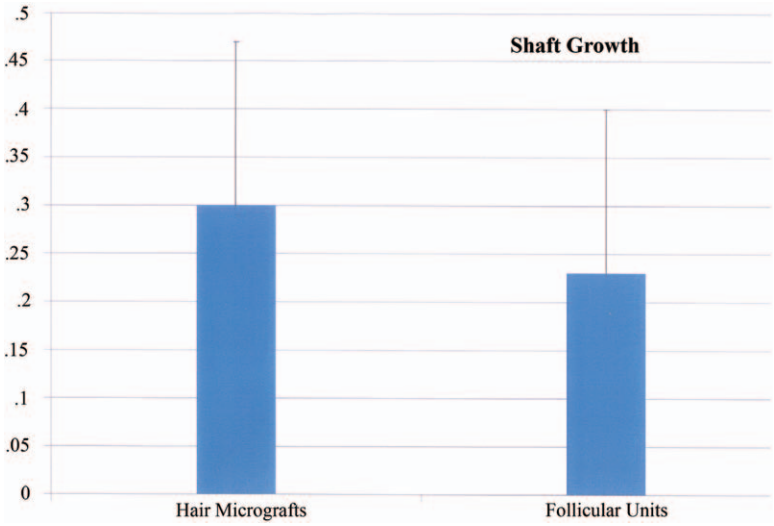

Fig. 3. Statistically significant difference between the growth rate of hair micrografts (mean 10-day growth rate = 0.30 mm) and that of follicular units (mean 10-day growth rate = 0.23 mm).

DISCUSSION

With this study, we compared survival and growth rates of both human hair FUs and conventional one-hair micrografts by means of a reliable and consistent in vitro system. The purpose of FUs, all along, has been to increase hair density, while maintaining a natural appearance of the scalp. Nowadays, all surgeons do not use the same procedures to perform hair transplantation[14–17] due to the lack of a generally recognized technique. Several procedures have been depicted and classified[2] on the resilience of donor area, graft harvest, recipient site, graft insertion, and distribution. It is essential, both in micrografting and in FU transplantation, to keep all FUs unaltered, when dissected; as highlighted by Greco et al,[18] the smaller the graft, the more notable the harm caused by squeezing. Hence, a small amount of tissue surrounding the follicle seems to play a pivotal role in preserving the graft from damage. On the other hand, an appreciable volume of adipose tissue enclosing the follicle incorporated in the graft may result in a deficient sustenance supply to follicular cells, perhaps decreasing shaft-graft yield; our data somewhat strengthened this speculation, illustrating a shorter shaft growth of FUs compared with that of conventional hair micrografts. In consonance with this supposition, the thinner the tissues enclosing FUs, the more encouraging graft growth appears to be.

CONCLUSION

The fact that this study was carried out in an in vitro system must unquestionably be taken into account and there may, therefore, be further elements acting as a pivotal role in graft survival and in vivo growth.

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