# The Effects of Dehydration, Preservation Temperature and Time on the Hair Grafts

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***Background*** **: Careful manipulation of hair grafts is essential for a good yield of transplanted hair.**

***Objective*** **: The aim of this study was to evaluate some of the factors responsible for poor graft yield, such as dehydration of the graft and the temperature and duration of preservation.**

***Methods*** **: First, for the dehydration study, isolated single hair follicles were left on dry gauze for 0, 5, 10, 20, and 30 minutes at room temperature. Secondly, to evaluate the effect of preservation temperature and time on the hair graft, follicles were preserved in saline for 5 minutes as a control, then for 6, 24, and 48 hours both at room temperature and at 4℃, respectively. Viability of preserved follicles was judged based on organ culture.**

***Results*** **: Elongation of hair follicles was seen in 96 %, 94 %, 94 %, 83 %, and 68 % for 0-, 5-, 10-, 20-, and 30-minute air-exposed groups, respectively. Survival was seen in 95%, 92%, 40% and 34% at room temperature and 96%, 94%, 76% and 50% at 4℃ for follicles preserved in saline for 5 min (control), then for 6, 24, and 48 hours, respectively.**

***Conclusion*** **: We suggest that, along with careful manipulation of hair units, high survival can be achieved with the avoidance of graft dehydration and preservation of the grafts at low temperatures if the operation time extends for more than 6 hours.**

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*Key Words* : Dehydration, Hair transplantation, Preservation temperature and time

Micrografts are often used for the treatment of male pattern baldness and give excellent cosmetic results. However, this technique, results in poor growth due to the increased fragility of follicular units[1]. Most of the reports on poor hair growth somehow implicate the rough handling of follicular units during dissection and the extended time of dissection spent on long transplant sessions[2-3]. The aim of this study was to evaluate, in an organ culture method[4], factors responsible for poor graft yield, such as the dehydration of grafts and/or the temperature and length of preservation in order to enhance graft survival for the surgery of hair transplantation.

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## MATERIALS AND METHODS

Upon obtaining informed consent from each patient, occipital scalp samples were obtained from 20 healthy male pattern baldness patients (ages 29-45 years, mean 38) at surgery for hair transplantation. Isolation of anagen hair follicles was achieved by using a surgical blade. A total of 1300 anagen hair follicles (65 follicles/person) were obtained: 500 for the dehydration study and 800 for the

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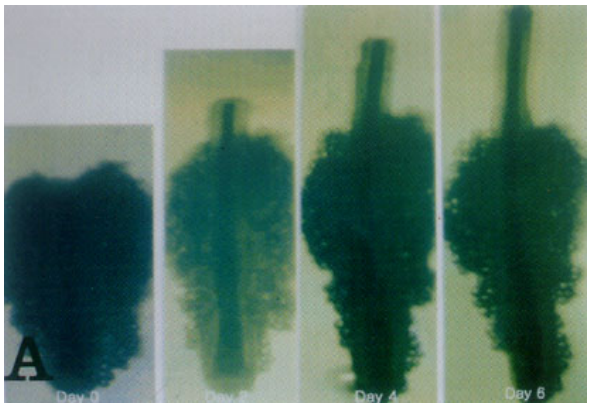


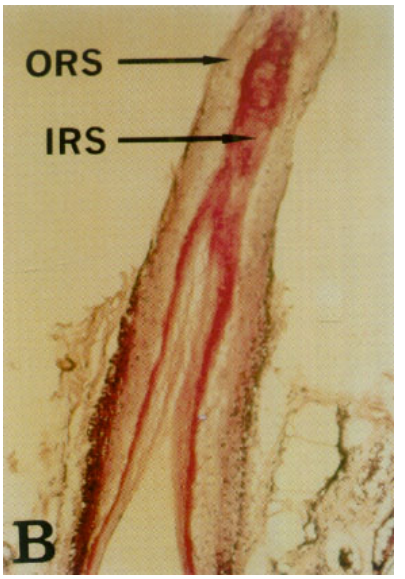


**Fig. 1.** A. Growing appearance of the hair follicle at fresh isolated (Day 0), 2 days (Day 2), 4 days (Day 4) and 6 days (Day 6) after organ culture. B. It showed elongation of hair follicle. ORS, outer root sheath; IRS, inner root sheath.

preservation temperature and time study. In order to observe growth of the hair shaft and the outer root sheath in vitro, hair follicles were transected just below the sebaceous gland and lower parts of follicles were used. For the dehydration study, 100 lower parts of the follicles each were left on dry gauze for 0, 5, 10, 20, and 30 minutes at room temperature and organ culture followed.

To evaluate the effect of preservation temperature and time on the hair graft, 100 follicles each were preserved in saline for 5 minutes as a control, then for 6, 24, and 48 hours both at room temperature and at 4℃, respectively. All the follicles for dehydration study and temperature study were cultured for 7 days in 500 $\mu$l of Williams E medium containing the following supplements: 1% fetal calf serum, 10 ($\mu$l /ml transferrin, 10 $\mu$l/ml insulin, 10 ng/ml sodium selenite, 10 ng/ml hydrocortisone, 100 U/ml penicillin, 100 $\mu$g/ml streptomycin, 2.5 $\mu$g/ml fungizone. Supplemental medium was prepared fresh before each experiment and changed every 72 hours. Follicles were maintained free-floating in individual wells of 130-well plates in an atmosphere of 37℃, 5% $CO_2$/95% air, and 100% humidity. The length of each follicle was measured microscopically at a magnification of 20 X immediately after storage in the medium and at the end of the 7-day culture period. Follicles that did not show elongation or had lost normal follicular architecture due to degeneration late in the culture period were regarded as not survived.

## RESULTS

Living hair follicles showed elongation of the hair shaft as well as the outer root sheath (Fig.1A, 1B). Elongation of hair follicles was seen in 96 %, 94 %, 94 %, 83 %, and 68 % for the 0, 5, 10, 20, and 30 minute air-exposed groups, respectively (Table 1). Grafts exposed to air for 10 minutes or less showed no difference in their survival. On the other hand, survival rates significantly decreased in follicles dehydrated for 20 minutes or more.

Hair follicles preserved in petri dishes with saline for 6 hours at room temperature showed 92 % survival, compared with 94% survival at 4℃. Thus, there is no difference in the survival rate regardless of whether hair follicles are preserved at room temperature or 4℃ within 6 hours after harvesting the donor scalp. In contrast, there is a significant difference between the survival rate of hair follicles preserved for 24 hours or more at room temperature and those preserved at 4℃ (Table 2).

## DISCUSSION

Follicular unit transplantation has recently been developed for the treatment of male pattern baldness.

**Table 1.** Effect of dehydration on hair grafts

| Duration of air-exposure (min) | Survival rate (%) |
|---|---|
| 0 (No exposure to air) | 96 |
| 5 | 94 |
| 10 | 94 |
| 20 | 83* |
| 30 | 68* |

* $p < 0.05$

minutes or more showed a significant decrease in survival rate. Therefore, we can assume that even though a brief dehydration can influence the cellular structure, it does not mean poor growth directly. However, the follicle must be kept moist in order not to disrupt the growth of the hair graft.

Preserving the grafts at a low temperature is generally recommended in order to enhance survival rate of the grafted hairs[7]. Knowing the best way to preserve hair grafts may be important because with the advent of megasessions, a significant period of time may elapse between graft harvesting and implantation. In this study, within 6 hours after harvesting, there is no significant difference in the survival of preserved hair grafts between room temperature and 4℃, which was consistent with the study by Raposio et al[8]. showing 87 % and 88 % survival of grafts preserved for 5 hours at room temperature, at 4℃, respectively. Our findings are also consistent with the in vivo study reported by Limmer[1], who stored the grafts in saline at 4℃. Therefore, in our opinion, there is no benefit provided by cold-storing the grafts within a 6 hour time period since the same growth results are achieved at room temperature. However, preserving grafts at 4℃ is better than storing at room temperature if the follicles are required to be preserved for more than 6 hours. In conclusion, preventing graft dehydration and preserving the grafts at a lower temperature if the operation time lasts for more that 6 hours will optimize graft survival, in addition to careful manipulation of hair units.

**Table 2.** Effect of preservation temperature and time on hair grafts

| Duration of preservation (hrs) | Survival rate (%) | |
|---|---|---|
| | Room temperature | 4℃ |
| Control | 95 | 96 |
| 6 | 92 | 94 |
| 24 | 40* | 76 § |
| 48 | 34* | 50 § |

*, § $p < 0.05$

The result of this technique is much better than that of previous methods such as punch grafts, and gives a natural looking appearance. However, dissecting hundreds or thousands of follicular units is an extremely tedious and time-consuming job and introduces a new set of problems, all potentially manifesting in poor growth. Shiell and Norwood[5] first described the X-factor in 1984 as something producing unexpected and unexplained poor growth in 4-mm grafts from one surgeon to another. Greco[2-3], in 1994, greatly increased our awareness of the problems in dealing with small grafts with his introduction of the term "H-factor" to describe iatrogenic contributions to poor growth. Greco focused on mechanical trauma as a major culprit, offering the logical explanation that as grafts became smaller, they would be more subject to a host of insults that included crushing, squeezing, bending, drying, and warming. When isolating hair grafts, drying the hair follicle is easier than keeping it moist. It is generally agreed upon that excessive drying due to air exposure may be one of the causes of poor growth. Gandelman, et al[6]. observed microscopic damage, for instance, clear nuclear and cytoplasmic alterations in hair follicles which were allowed to dry on surgical gloves for about 3 minutes. On the other hand, in our experiment, dehydration of the follicles for 10 minutes did not show any difference in graft survival compared with the control. However, dehydration for 20

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