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ORIGINAL ARTICLE

Donor hair follicle preservation by partial follicular unit extraction. A method to optimize hair transplantation

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Abstract

Background: There are different stem cell pools located in the hair follicle. *Objective*: To try to determine whether follicular units can survive a partial extraction and whether this partial extracted follicular unit can regenerate new hairs. *Methods*: From five individuals, between 100 and 150 grafts were harvested from the occipital area of the scalp. Suitable grafts were implanted into the recipient area. Hair growth and characteristics in the donor area and the recipient area were observed at different intervals. *Results*: After 3 months, between 92.1% and 104.1% (mean 97.7%) of the partial follicular units in the donor sites survived and produced hairs with the same characteristics. After 1 year, 91.1–101.7% (mean 95.9%) of the implanted partial follicular units regenerated hair growth with the same characteristics as the hairs in the donor area. *Conclusions*: We revealed that extracted partial longitudinal follicular units transplanted to the recipient area can be used as complete follicular units to regenerate completely differentiated hair growth with the same characteristics as in the donor area. We also revealed that the partial follicular units in the donor area can survive and produce the same number of hairs with the same characteristics. This technique enables us to generate two hair follicles from one follicle with consistent results and preserve the donor area.

Key words: *alopecia, androgenetic alopecia, esthetics, hair restoration, hair transplantation, humans*

Introduction

Over 60% of men and 50% of women suffer from androgenetic alopecia (1,2). Since this type of hair loss is a semi-natural process, and medication can only inhibit this temporarily, hair transplantation is the only method to restore hair permanently.

There are different techniques of hair transplantation, all with their advantages and disadvantages. The most common and known hair transplantation method is the so-called 'strip' method (3). A strip of skin containing hair follicles is removed, cut into grafts and implanted in the recipient area. In recent years, new methods have developed, of which the most promising is the follicular unit extraction (FUE) method (4). With this method, whole follicle units are extracted one by one and implanted one by one back into the recipient area. The FUE method is a major step towards perfecting hair transplantation. Although the FUE method is more patient friendly and leaves only tiny scars compared to the strip method, which leaves visible linear scars at the donor area, the major disadvantage of both methods is that the extracted hair follicles are removed and the source of potential grafts will be consumed in time. Hair transplantations with the described methods will always be limited by the availability of donor hair follicles because no re-grow will occur in the donor area.

The cosmetic result depends not only on the graft type (single-hair grafts or follicular units), the survival rate of the transplantation and the skill of the surgeon, but also on the number of grafts one can transplant.

To date, no multiplication of human hair follicles in vitro is possible. The only theoretical way to preserve a significant part of the donor hair follicles could be

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Table I. Selection of the grafts.

Number a	Number of grafts					
	b	c	d	e	f	g
	Extracted	Complete follicular units	Partial longitudinal follicular units – suitable	Incomplete follicular units – unsuitable	Total number of visible hairs of the suitable grafts	Total number of visible hairs of the unsuitable grafts
1	125	0	110 (88%)	15 (12%)	238	20
2	150	0	124 (83%)	26 (17%)	267	40
3	150	0	104 (69%)	46 (31%)	197	70
4	125	0	105 (84%)	20 (16%)	230	25
5	100	0	94 (94%)	6 (6%)	203	13
Mean	130	0	107.4 (83.6%)	22.6 (16.4%)	227	33.6

partial FUE. This idea is not unrealistic and is supported by different experiments (5,6). Kim and Choi (5) found that, in humans, the proximal part of the hair follicle cannot regenerate into a differentiated hair follicle, but the distal part of the follicle can, eventually resulting in a fully developed hair follicle (7). Reynolds et al. found that, although the dermal papillae of humans cannot induce new hair growth, the sheath of the lower part of the hair follicle can (6). These apparently contradictory results indicate that both proximal and distal areas of the hair follicle should contain follicular stem cells that can induce hair growth.

In an earlier study we revealed that hair follicles from skin biopsies expressed CK19 and Bcl-2 in cells from the upper to the lower third of the follicle (8). Commo et al. also observed that distinct areas in the hair follicle from skin biopsies are positive for CK19 (9). The fact that these areas are also Bcl-2 positive and Bax negative is a strong indication for different follicular stem cell sites which can induce hair growth. Positivity for CK19 and Bcl-2 corresponds to infrequent cell division in these areas, as concluded from the absence of Ki-67 staining (10,11). The fact that these cells are positive for Bcl-2 and CK 19, but Ki-67 and Bax negative, is a strong indication that they represent follicular stem cells in the hair follicle.

In case of partial longitudinal FUE, where follicular stem cells remain at the donor site as well as in the partial extracted follicle, a donor site capable of multiple hair transplantations should become possible.

The main objectives of this study are: (i) to determine the percentage of re-growth and characteristics of hairs from transplanted partial longitudinal follicular units in the recipient area; and (ii) to determine the survival rate and the percentage of re-growth of the partial follicular units remaining in the donor area; the characteristics of the re-grown hairs are also evaluated.

Materials and methods

Patients

Five healthy male individuals (aged 36–61 years, mean 44.8 years) (Table I) who consulted the Hair

Table II. Re-growth of the hairs in the donor area.

Number a	Time b	Number of visible hairs in outlined donor area			Re-growth in the donor area		
		c	d	e	f	g	h
	Duration of the extraction in minutes (graft/min)	Before extraction	Directly after extraction	12 months after extraction	Total number of visible hairs of the suitable grafts	Total number of re-grown hairs	Percentage re-growth in the donor area (%)
1	75 (1.7)	370	112	354	238	222	93.3
2	95 (1.6)	392	85	401	267	276	103.4
3	80 (1.9)	344	77	352	197	205	104.1
4	65 (1.9)	319	64	308	230	219	95.2
5	75 (1.3)	318	102	302	203	187	92.1
Mean	(1.68)	348.6	88	343.4	227	221.8	97.7

Figure 1. Close-up of the triple-waved tipped extraction needle.

Science Institute with proven androgenetic alopecia and gave their informed consent, participated in the study. The protocol was approved by the Institutional Review Board and the study was conducted according to the declaration of the Helsinki principles.

The technique

a. Preparation and outlining of the donor site. On the occipital side of the scalp, an area of 15 × 5 cm was shaved, disinfected with chlorhexidine 2% lotion and anaesthetized with lidocaine 2% with adrenaline (AstraZeneca). Within that area, 1.5 × 1.5 cm was outlined with an acupuncture needle dipped in semi-permanent black pigment.

b. Counting of the hairs. The outlined area was photographed with a digital camera (Nikon E4800), and the hairs in the area were counted (Table II, column c).

c. Extraction of the partial longitudinal follicular units (grafts). At least 100 grafts were harvested with hollow triple-waved-tipped, partially blunt needles with an inner diameter of 0.6 mm (Figure 1) (Hair Science Institute®, Amsterdam, The Netherlands) (Table I, column b). To extract a partial longitudinal follicular unit, we used the hair shafts as guidance for the needle. This enables us to extract a partially longitudinal follicular unit, even when the follicular unit is not in a perfect triangular configuration.

Figure 2A shows a follicular unit containing the visible hairs (brown), hair follicle (dark pink) and connective tissue (white). The needle is placed around the visible hairs and rotated until the grafts are detached from the dermis (Figures 2A and B). The grafts are extracted with micro-surgical forceps (Figure 2C). The aim of the extraction is to remove only a part of the follicle unit, containing follicle and connective tissue from several hair follicles, and leave sufficient follicle unit tissue behind to regenerate hairs (Figures 2D and E). After the extraction, Fucidin

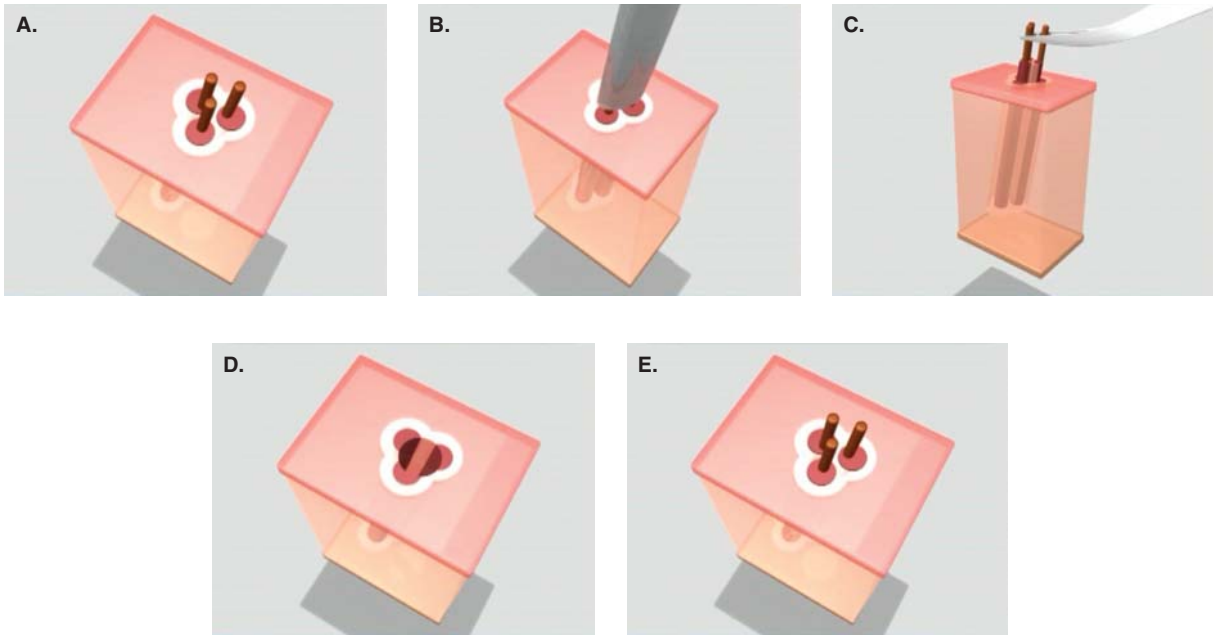

Figure 2. Illustrations of the procedure in the donor area. (A) The whole follicular unit. (B) Extraction of the longitudinal partial follicular unit with a 0.6 mm needle. (C) Extraction of the longitudinal partial follicular unit with micro-surgical forceps. (D) Part of the follicular unit which is left behind. (E) Re-growth in the donor area.

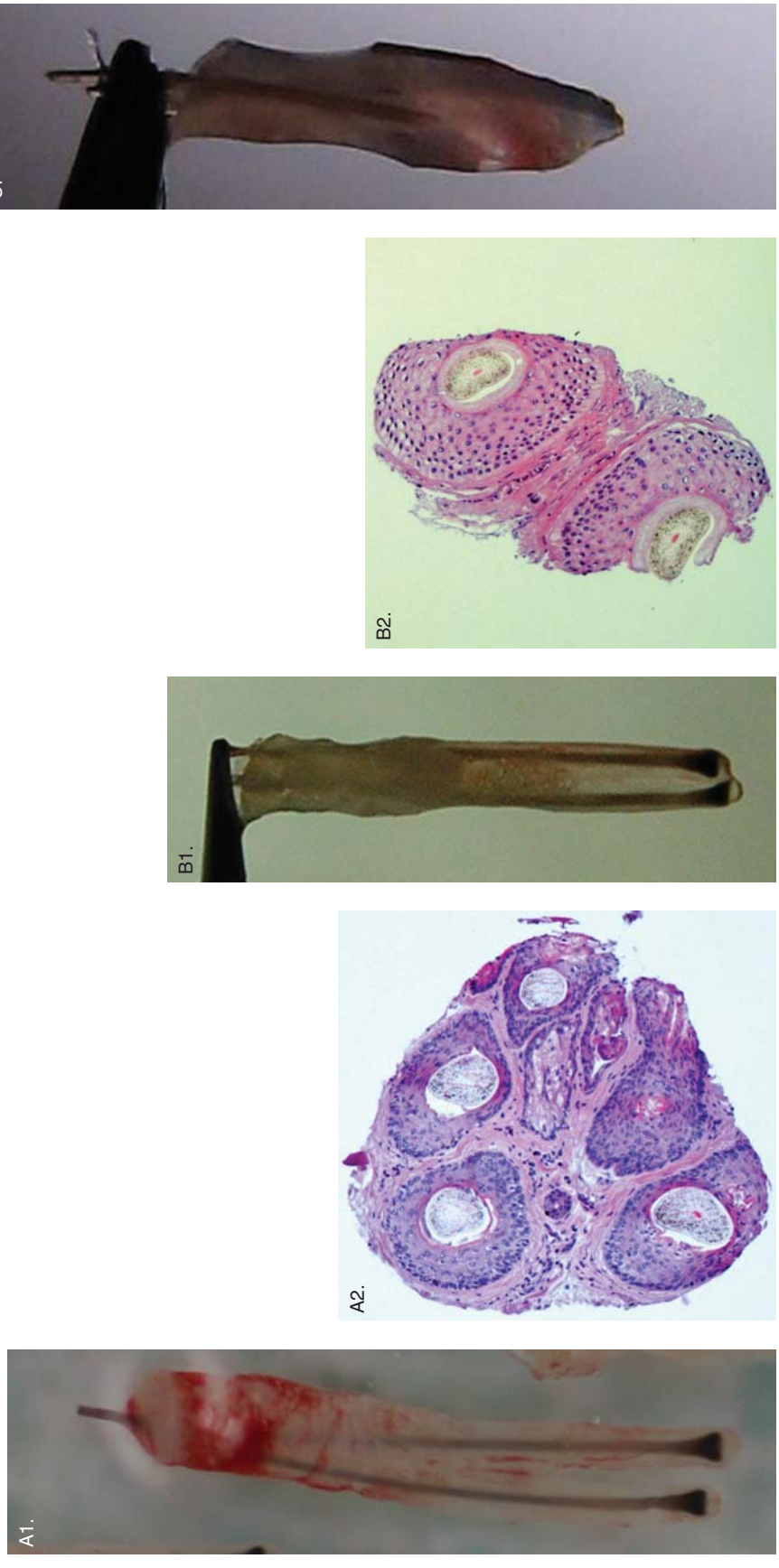

Figure 3. The grafts. (A1) Complete follicular unit grafts. (A2) A haematoxylin & eosin (HE)-stained transversal sectioned complete follicular unit (10×). (B1) Partial longitudinal follicular unit grafts. (B2) A haematoxylin & eosin (HE)-stained transversal sectioned partial longitudinal follicular unit (20×), where a considerable part of the follicular unit is left behind at the donor site. (C) Grafts which contain insufficient tissue.

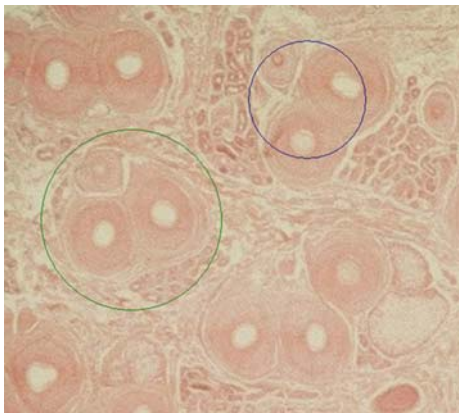

Figure 4. The difference between a complete follicular unit (green circle) and a partial longitudinal follicular unit (blue circle) in a transversal section of the skin.

Cream® (Leo Pharma, Breda, The Netherlands) was applied on the donor area.

d. Selection of the grafts. After the extraction, the grafts were visually evaluated, selected and divided in three groups: complete follicular units (Figure 3A1) (Table I, column c); partial longitudinal follicular units (suitable grafts) (Figure 3B1) (Table I, column d); and incomplete follicular units (unsuitable grafts), which did not contain sufficient tissue (Figure 3C) (Table I, column e). By using a magnifier (2×) we were able to distinguish between partial longitudinal follicular units, which show terminal hairs at the border and do not contain surrounding tissue, and complete follicular units, which contain complete hair follicles with surrounding tissue. We illustrate the difference between a complete follicular unit (Figure 3A1) (Table I, column c) and a partial longitudinal follicular unit (suitable grafts) (Figure 3B1) (Table I, column d) with Figure 3A2, which shows a haematoxylin & eosin (HE)-stained transversal sectioned complete follicular unit (10×), and Figure 3B2, which shows a HE-stained transversal sectioned partial longitudinal follicular unit (20×), where a considerable part of the original follicular unit is left behind at the donor site. Figure 4 illustrates the difference between a complete follicular unit (green circle) and a partial longitudinal follicular unit (blue circle) in a transversal section of the skin.

Suitable longitudinal partial follicular units (Figure 3B1) were visually selected (Table I, column d) and stored in the preservative medium for 2 hours until implantation. The medium is composed of the following ingredients: sodium chloride, potassium chloride, magnesium sulphate, sodium phosphate, calcium chloride, glucose, sodium bicarbonate, sodium lactate, sodium pyruvate, human serum albumin, insulin, bis(maltolato)oxovanadium (BMOV) and α-tocopherol (vitamin E) (Hair Science Institute).

e. Counting the number of hairs within the suitable and unsuitable grafts. The total number of visible hairs in the selected partial longitudinal follicular units (suitable grafts) (Table I, column f), as well as the total number of visible hairs in the unsuitable incomplete follicular units, was counted (Table I, column g).

f. Measurement of the hair diameter from hairs derived from the donor area. From the suitable grafts, 10 hairs were measured with an electronic digital micrometer to determine their diameter (Table III, column b).

g. Measurement of the hair diameter from hairs in the donor area. After 12 months, 10 hairs in the outlined donor area were measured with an electronic digital micrometer to determine their diameter (Table III, column c).

h. Preparation and outlining the recipient area. The recipient area was disinfected with chlorhexidine 2% lotion and anaesthetized with lidocaine 2% with adrenaline (AstraZeneca). Within this area, 2.5 × 2.5 cm was outlined with an acupuncture needle dipped in semi-permanent black pigment. Miniscule holes were made with a hollow needle with an inner diameter of 0.6 mm (Hair Science Institute). Remaining bald tissue was removed with micro-surgical forceps (Figure 5A).

i. Implantation of the grafts. After preparation of the recipient area, the selected grafts were implanted with micro-surgical forceps (Figure 5B) (Table IV, column b). The aim of the implantation is to implant sufficient follicle and connective tissue from several hair follicles to regenerate hair growth (Figure 5C).

Table III. Characteristics of the hairs in the donor area.

Number	Average diameter of 10 hairs	
a	b Before extraction (μm)	c 12 months after the extraction (μm)
1	55	54
2	94	93
3	56	55
4	82	80
5	65	63
Mean	70.4	69

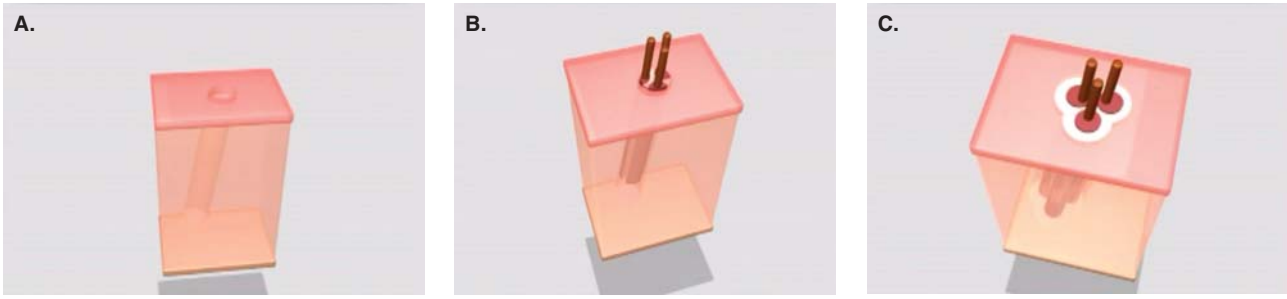

Figure 5. Illustrations of the procedure in the recipient area. (A) Holes in the recipient area, made with a same sized needle. (B) Implantation of the longitudinal partial follicular unit with micro-surgical forceps. (C) Re-growth in the recipient area.

j. Measurement of the hair diameter in the recipient area. After 12 months, 10 hairs in the outlined recipient area were measured with an electronic digital micrometer to determine their diameter (Table V, column c).

Evaluation, calculation and follow-up

a. Evaluation and calculation of re-growth in the donor area. The outlined area at the donor site was photographed before the extraction (Figure 6A) and the visible hairs in the outlined area were counted before and directly after the extraction (Figure 6B) (Table II, columns c and d). At intervals of 1 week (Figure 6C), 3 and 12 months after the extraction, the outlined area was shaved and photographed. After 12 months, the visible hairs in the outlined area were also counted (Table II, column e).

The number of re-grown hairs in the donor area (Table II, column g) was calculated as follows: the total number of visible hairs after 12 months in the outlined area (Table II, column e) minus the number of hairs which were left in the donor area (Table II, column d) minus the total number of visible hairs in the unsuitable incomplete follicular units (Table I, column g).

The survival rate in the donor area (Table II, column h) was calculated as follows: since the total number of visible hairs in the suitable grafts (Table II, column f) are supposed to be the hairs which are 'extracted' and suitable to regenerate new hairs and the total number of re-grown hairs in the donor area (Table II, column g) are supposed to be the hairs which have been preserved after extraction, the survival rate in the donor area (Table II, column h) was calculated as follows: *the total number of re-grown hairs in the donor area* (Table II, column g) *divided by the total number of visible hairs in the suitable grafts* (Table II, column f).

b. Evaluation of the characteristics in the donor area. From the suitable grafts, the diameter of 10 hairs was measured with an electronic digital micrometer and the average calculated (Table III, column b). Twelve months after the extraction, the diameter of 10 hairs in the same area was measured again by an electronic digital micrometer and the average calculated (Table III, column c).

c. Evaluation and calculation of re-growth in the recipient area. The outlined recipient area was photographed before and at intervals of 1 week, and 3 and 12 months after implantation. The visible hairs in the outlined area were counted before (Table IV, column d) and 12 months after the implantation (Table IV, column e).

Table IV. Re-growth of the hairs in the recipient area.

Number		Number of visible hairs in outlined recipient area			Re-growth in the recipient area		
a	b	c	d	e	f	g	h
	Partial longitudinal follicular units – implanted	Implantation time in minutes (graft/min)	Before implantation	12 months after implantation	Total number of visible hairs of the suitable grafts	Total number of re-grown hairs	Percentage re-growth in the donor area (%)
1	110	21 (5.2)	37	279	238	242	101.7
2	124	25 (5.0)	52	314	267	262	98.1
3	104	17 (6.1)	70	263	197	193	98.0
4	105	21 (5.0)	47	253	230	206	89.6
5	94	15 (6.2)	62	247	203	185	91.1
Mean	107.4	19.8 (5.5)	53.6	271.2	227	217.6	95.9

Table V. Characteristics of the hairs in the recipient area.

Number a	Average diameter of 10 hairs	
	b Before extraction (μm)	c 12 months after implantation (μm)
1	55	53
2	94	91
3	56	55
4	82	80
5	65	66
Mean	70.4	69

The number of re-grown hairs in the outlined recipient area (Table IV, column g) was calculated as follows: *the total number of visible hairs after 12 months in the outlined area* (Table IV, column e) *minus the number of hairs which was already present in the recipient area* (Table IV, column d).

The survival rate in the outlined recipient area (Table IV, column h) was calculated as follows: the total number of re-grown hairs in the outlined recipient area (Table IV, column g) divided by the total number of visible hairs in the suitable grafts (Table IV, column f).

d. Evaluation of the characteristics in the recipient area. The diameter of 10 hairs in the recipient area was measured by a micrometer 12 months after implantation and the average calculated (Table V, column c). This was compared with the average diameter of 10 hairs from the extracted partial follicular units (Table V, column b).

e. Multiplication of hairs. The number of extra hairs, and therefore the multiplied hairs (Table VI, column f) was calculated as follows: *the number of re-grown hairs in the donor area* (Table VI, column c) *plus the number of re-grown hairs in the outlined recipient area* (Table VI, column d) *minus the total number of visible hairs in the suitable grafts* (Table VI, column e).

The multiplication rate (Table VI, column g) can be calculated in different ways: the number of extra (multiplied) hairs (Table VI, column f) divided by the total number of visible hairs in the suitable grafts (Table I, column e) or the percentage of re-grown hairs in the donor area (Table II, column h) plus the percentage of re-grown hairs in the outlined recipient area (Table IV, column h) minus 100%.

Results

Grafts

In this study of five patients, between 100 and 150 grafts (mean 130 grafts) per patient were extracted (Table I, column b). There were no grafts that contained complete follicular units (Table I, column c). Between 69% and 94% (mean 83.6%) of the extracted grafts contained partial follicular units and therefore were suitable to be implanted in the recipient area (Table I, column d). The suitable grafts contained between 197 and 267 visible hairs (mean 227 hairs) (Table I, column f). Between 6% and 31% (mean 16.4%) of the extracted grafts were not used for implantation in the recipient area (Table I, column e). Unsuitable grafts contained between 13 and 70 visible hairs (mean 33.6 hairs) (Table I, column g).

Re-growth of the hairs in the donor area

The extraction time varied between 1.3 and 1.9 grafts (mean 1.68 grafts) per minute (Table II, column b). The number of hairs in the outlined area before extraction varied between 318 and 392 hairs (mean 348.6) (Table II, column c) and between 64 and 112

Table VI. Multiplication of the hairs.

a	b	c	d	e	f	g
Number	Hairs left in the donor area	Number of re-grown hairs in the outlined donor area	Number of re-grown hairs in the outlined recipient area	Total number of visible hairs of the suitable grafts	Multiplication	Multiplication rate (%)
1	132	222	242	238	226	95
2	125	276	262	267	271	101.5
3	147	205	193	197	201	102.1
4	89	219	206	230	195	84.8
5	115	187	185	203	169	83.2
Mean	121.6	221.8	217.6	227	212.4	93.3

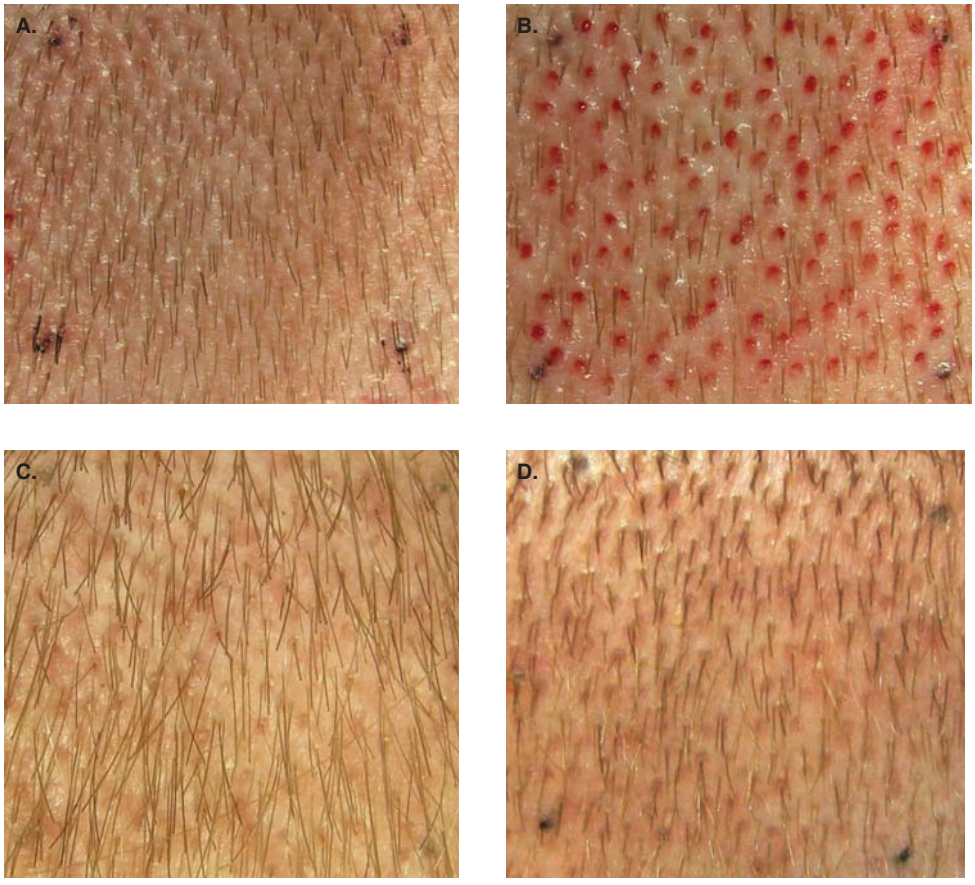

Figure 6. Evaluation of the donor area. (A) The outlined area at the donor site before the extraction. (B) The outlined area at the donor site directly after the extraction. (C) The outlined area at the donor site 9 days after the extraction. (D) The outlined area at the donor site 1 month after the extraction.

hairs (mean 88 hairs) were visible directly after extraction (Table II, column d). Twelve months after extraction, between 302 and 401 hairs (mean 343.4 hairs) were visible in the outlined donor area (Table II, column e).

If we assume that the number of hairs in the outlined area will remain the same, the percentage of re-grown hairs varies between 92.1% and 104.1% (mean 97.7%) (Table II, column h).

The close-up picture of the donor site 1 week after extraction (Figure 7) shows re-growth in the donor area. The circles most probably show where the grafts were harvested since the area around the hairs is pinkish and these hairs are shorter compared with the surrounding hairs. The influence of extraction of unsuitable grafts (Table I, column g) on the re-growth is minimal, since the number of unsuitable grafts is considerably smaller than the suitable grafts (Table I, column f). In the blue circles small hairs are growing out. These circles show the re-growth of the hairs in the donor graft sites. The red circles do not contain visible hairs. However, in these donor-graft sites, small black points are visible. Evaluation after 3 and 12 months revealed that there were no bald spots visible anymore. This could mean that too much tissue had been taken to reveal re-growth within 1 week but that re-growth would be visible in successive weeks. Figures 6A–D, where the outlined area at the

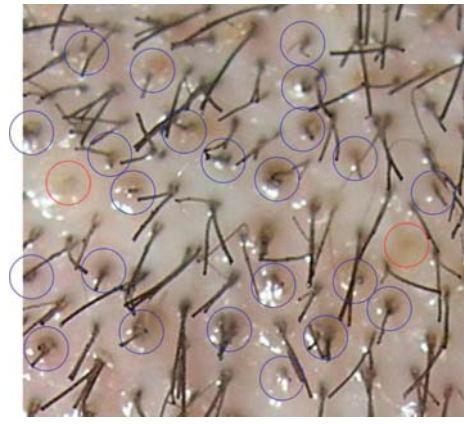

Figure 7. Close-up picture of the donor-site one week after extraction of the longitudinal parts of the follicular units. The pink spots shows where the grafts were taken. In these pink spots small hairs growing out (blue circles) are visible. This shows the re-growth of the hairs in the donor graft sites. Some pink spots do not contain small hairs (red circles). In these donor-grafts sites, probably too much tissue is taken to reveal re-growth after a week. However, there is a possibility that there will be re-growth after months.

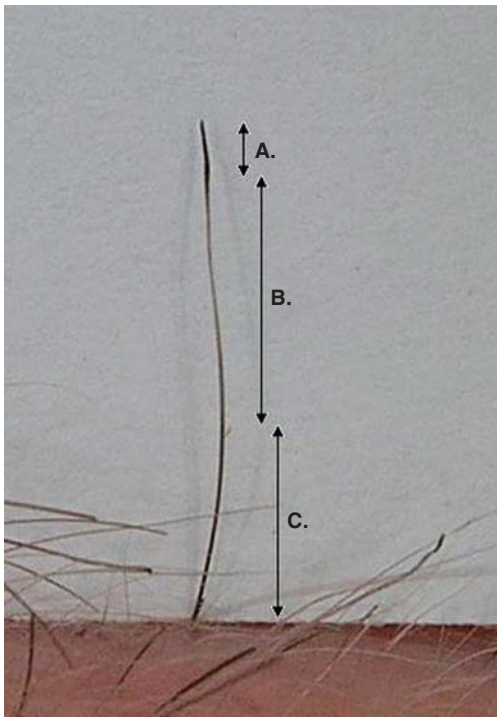

Figure 8. A re-grown hair in the recipient area after 3 months. This picture shows that after implantation, the partial follicular unit is able to produce a differentiated hair, but in the first period after implantation, the re-grown hair is thinner than normal (part b); after 3 months this hair has developed into a hair with the same characteristics, such as the diameter (part c) in the donor area (part a).

donor site has been evaluated before (Figure 6A), directly after (Figure 6B), 1 week after (Figure 6C) and 1 month after (Figure 6D) the extraction, confirmed this. The average hair diameter of the patients varied between 55 and 94 μm (mean 70.4 μm) (Table III, column b). After 12 months, the average hair diameter of the patients varied between 54 and 93 μm (mean 69 μm) (Table III, column c).

Re-growth of the hairs in the recipient area

Between 94 and 124 (mean 107.4) suitable grafts were implanted in the outlined recipient area (Table IV, column b). The implantation time varied between 5.0 and 6.2 grafts (mean 5.5 grafts) per minute (Table IV, column c). The number of hairs in the outlined area before implantation varied between 37 and 70 hairs (mean 53.6) (Table IV, column d) and 12 months after implantation between 247 and 314 hairs (mean 271.2 hairs) were visible in the outlined recipient area (Table IV, column e). Assuming that the number of hairs in the outlined area remains the same, re-growth in the outlined recipient area would be between 89.6% and 101.7% hairs (mean 95.9%) (Table IV, column h).

Characteristics of the hairs in the recipient area

The average hair diameter of the patients varied between 55 and 94 μm (mean 70.4 μm) (Table V, column b). After 12 months, the average hair diameter of the patients varied between 53 and 91 μm (mean 69 μm) (Table V, column c).

Most small hairs from the implanted grafts fall out during the first weeks after implantation, although some hairs are able to continue growing. Figure 8 does not show re-growth in the recipient area in general, but demonstrates the dynamic re-growth process of a partial follicular unit in the recipient area during the first 3 months after implantation. The partial follicular unit is able to produce a differentiated hair, initially. The re-grown hair is thinner than normal (Figure 8, part b), but after 3 months the hair has developed into hair with the same diameter and visible characteristics (Figure 8, part c) as in the donor area (Figure 8, part a).

Multiplication of the hairs

If we assume that the number of hairs left behind in the donor area (Table VI, column b) were the visible hairs directly after the extraction (Table II, column d) plus the visible hairs in the unsuitable incomplete follicular unit grafts (Table I, column g), the number of hairs which are multiplied varied between 169 and 271 hairs (mean 212.4 hairs) (Table VI, column f). This means a multiplication rate between 83.2% and 102.1% (mean 93.3%) (Table VI, column g).

Statistical analysis

Since only five patients participated in this study, no statistical analysis was performed.

Discussion

The grafts

In our study, the diameters of the individual hair follicles of the patients were large. Therefore, the follicular units of these patients, containing at least two hairs, are larger than the diameter of the extraction needle (diameter of 0.6 mm) used for harvesting the grafts. As a result, in this study, there was not one graft extracted which contained a complete follicle unit.

Re-growth in the donor area

Figure 9 shows pictures of the donor area of a patient before (Figure 9A), directly after (Figure 9B) and

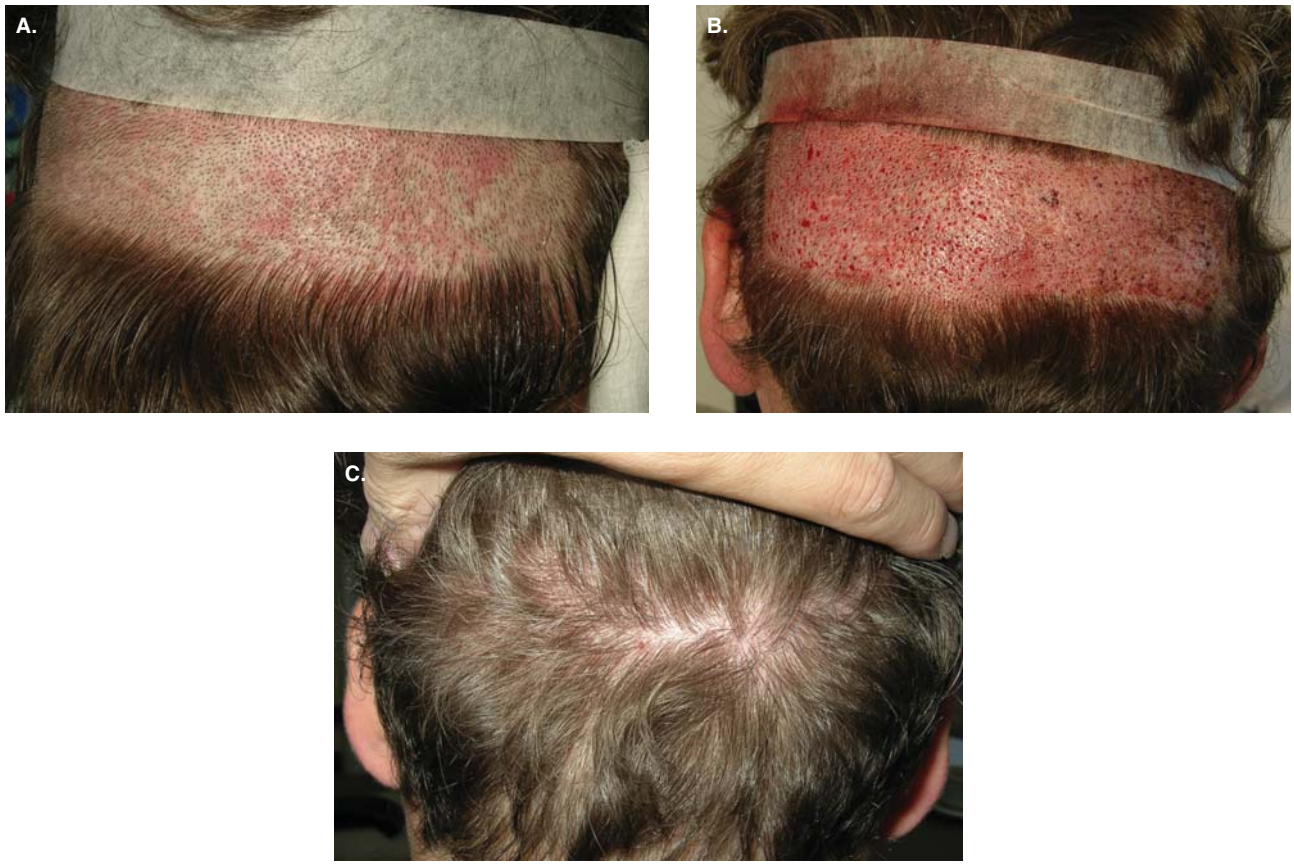

Figure 9. Photographs of the donor area. (A) The donor area of a patient before harvesting the grafts. (B) The donor area of a patient directly after harvesting the grafts. (C) The donor area of a patient 3 months after harvesting the grafts.

3 months after (Figure 9C) harvesting the grafts. After evaluation of the five patients in this study, almost all hair follicles in the donor site produced a hair after 12 months. In two cases, the number of hairs increased, probably due to invisible telogen hair follicles, which were not visible after extraction, but produced hairs in the successive period.

Since some hairs which were present after extracting the grafts were still present in the donor area when measuring the diameter after 12 months, these pre-existing hairs could also be measured instead of the hairs which had re-grown. Therefore, the average diameter after 12 months could be influenced by these pre-existing hairs. However, since the average diameter of the hairs in the donor area after 12 months was not reduced compared to the hairs in the grafts which had been measured, we could assume that the influence of the existing hairs is minimal.

Re-growth in the recipient area

After evaluation of the five patients, it was observed after 12 months that almost all implanted grafts produced a hair in the recipient site. In one case, the number of hairs increased, probably due to invisible telogen hair follicles, which were not visible directly after the implantation, but produced a hair a few months after implantation.

Since some hairs were present the recipient area before implantation, these pre-existing hairs could also be measured instead of the hairs from the grafts which had implanted. Therefore, the average diameter after 12 months could be influenced by these pre-existing hairs. However, since the average diameter of the hairs in the recipient area after 12 months was not reduced compared to the hairs in the grafts, which had been measured, and the number of pre-existing hairs was considerably lower compared to the implanted hairs, we could assume that the influence of the existing hairs is minimal.

Therefore, this study shows that:

(1) Extracted partial longitudinal follicular units containing viable follicular stem cells with connective tissue transplanted to the recipient area can be used as complete follicular units to regenerate completely differentiated hair growth with

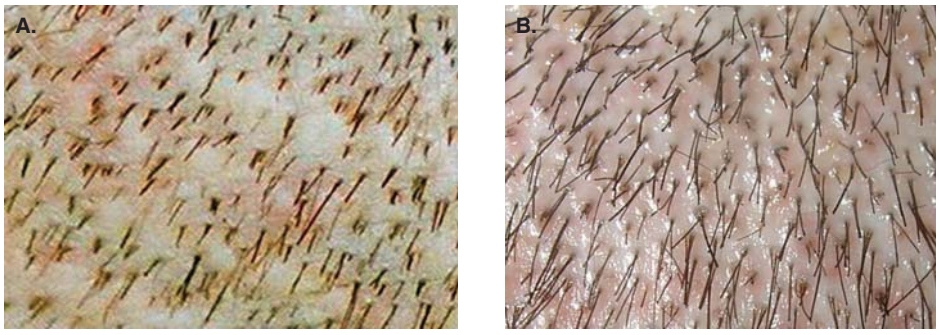

Figure 10. (A) Small scars in the donor area due to follicular unit extraction (FUE). (B) Close-up of the donor site 1 week after extraction of the longitudinal parts of the follicular units. The parts of the follicular units that remain in the dermis in the donor area produce hair with the same characteristics, such as the diameter.

the same diameter and characteristics as hair in the donor area.

(2) The partial follicular units which remain in the dermis in the donor area can survive and produce the almost same number of hairs with the same diameter and characteristics when a longitudinal part of this follicular unit is extracted.

Our clinical data are in correspondence with the findings of Kim and Choi (5) and Reynolds et al.(6). We have proven that two hair follicles can be generated from one as long as only a part of the follicle is dissected from the original source. However, it is essential to realize that harvested partial follicular units can be obtained as:

- transversal, as published by Kim and Choi (5)
- longitudinal, as proposed in this study.

It is also important to realize that, practically, harvesting partial follicular units with the preservation of the donor follicular units can only be obtained in large quantities if they are from the longitudinal type.

In this study, we were not able to perform immunohistochemical experiments because our group of individuals underwent a standard hair transplant procedure. However, from our and Kim's experiments, we can calculate the multiplication rate from the re-growth of the harvest follicular stem cells at the recipient site and re-growth of the left follicular stem cells at the donor site as follows: *the percentage of re-grown hairs in the donor area* (Table II, column h) *plus the percentage of re-grown hairs in the outlined recipient area* (Table IV, column h) *minus 100%*. These mathematical calculations are in full correlation with our clinical results.

Our clinical results concerning the re-growth of partial hair follicles are not in correlation with other clinical studies, such as the study of Er et al. Er et al. recommended not implanting sectioned hair follicle parts. They stated that the survival rate of the transected hair follicles is directly related to the level of transection and that the growth rate of the sectioned parts is not satisfactory and are thinner than the original follicles (12).

Earlier studies revealed that in conventional hair transplantation techniques, such as the strip-method, the preservative medium could influence the survival rate after implantation (13) and could reduce apoptosis in the grafts (14). The smaller the amount of tissue transplanted the more influence the preservative medium had on the viability and apoptosis of the transplanted tissue, the survival rate of the grafts and therefore hair growth. Since the amount of tissue in our grafts is considerably smaller than with conventional hair transplantation methods, the influence of the preservative medium could be important. We used a medium which contained anti-apoptotic compounds such as BMOV, anti-oxidants such as vitamin E and growth-stimulating factors. This could be one of the factors why, in this study, we were able to use only longitudinal parts of follicular units with a maximum diameter of 0.6 mm instead of whole follicular units to regenerate new differentiated hairs in the recipient area with the same characteristics, such as the diameter, as hair in the donor area.

If too much follicle tissue has been removed from the donor area, only the graft will regenerate a new hair. If insufficient follicle unit tissue is removed from the donor area, only this follicle unit in the donor area will be capable of producing hairs. Therefore, the amount of tissue extracted from the donor follicular unit is vitally important for hair growth in both donor and recipient area. To minimize the variability of the amount of tissue extracted, instruments such as conventional punch needles were not suitable as in most cases too much tissue was extracted (non-published data).

Besides the variability of the amount of extracted tissue, conventional punch needles also damaged the grafts. Since the amount of tissue in our grafts was considerably smaller than the conventional hair

transplantation methods, the damage to the grafts had considerably more influence on the survival rate of the grafts in our studies. Therefore, we had to develop new wave-tipped extraction needles of 0.5 and 0.6 mm (Figure 1). These needles use the hair shafts as a guide to extract longitudinal parts of the follicular unit which contain sufficient tissue to regenerate new differentiated hairs in the recipient area. The diameter of the grafts which contain the longitudinal parts of the follicular units extracted from the donor area varies between 0.5 mm and 0.6 mm. Since the diameter of a normal hair follicle is between 0.4 mm and 0.7 mm, and a follicular unit consists of at least two hair follicles, the needle is able to leave sufficient tissue behind to preserve the follicular unit in the donor area (Figure 4). Furthermore, this needle minimizes the damage to the grafts as well as the tissue in the donor area.

Differences with other transplantation techniques

The ideal hair transplantation should fulfil the following objectives:

(1) An excellent cosmetic outcome.
(2) 100% hair re-growth of the transplanted hair follicles.
(3) 100% preservation and therefore an endless source of donor hair follicles.
(4) No scarring.
(5) A safe and comfortable procedure.
(6) Short treatment duration.
(7) No recovery time.
(8) Not expensive.

Of all available transplantation techniques, there is no technique which fulfils all mentioned criteria. The main difference between our technique of partial longitudinal FUE compared with the other hair transplantation techniques is the preservation of the donor hair follicles without scarring.

In contrast to traditional hair transplantation techniques, which require a strip removal with a depth of 1–1.5 cm to obtain the hair follicles, we showed that successful transplantation is feasible using longitudinal partial follicular units with a diameter of 0.5–0.6 mm and 5–6 mm in length. Owing to minimal skin and tissue removal, there is minimal to no scarring, pain, or other post-surgical trauma such as nerve and vascular damage, nor is there the possibility that the linear scar will 'stretch out' over time. Absolutely no stitches or bandages are required.

FUE is another technique where the whole follicular unit is transplanted, without leaving sufficient tissue behind to regenerate a new hair or follicular unit. However, since the total follicular unit is extracted, this results in small scars in the donor area (Figure 10A).

With longitudinal partial follicular unit transplantation, parts of the follicular units remain in the dermis in the donor area. After longitudinal parts of these follicular units are extracted, they will survive and produce the same number of hairs with the same diameter and characteristics. These follicular units in the donor area can be used again in consecutive treatments (Figure 10B).

Another difference is the progression of hair growth. In traditional hair transplantation, the majority of implanted hairs would fall out quickly after implantation and re-growth would occur within 3–6 months. In our study, re-growth started 5–8 months after implantation, but could sometimes take more than 12 months. This phenomenon was revealed in other hair transplantation studies with dissected hair follicles (15).

The weakness of this study is the limited number of patients. We also included patients who had had previous (traditional) hair transplantation. Therefore, a larger group of patients is necessary to study the real clinical relevance of this technique.

Longitudinal partial follicular unit transplantation is a very labor intensive procedure and to transplant sufficient grafts takes a full day. This technique may represent the first reliable patient-friendly method to generate two hair follicles from one hair follicle with consistent results and preserve the donor area. This technique is therefore suitable for people with a very limited donor area, the most extreme example of this being burn victims.

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