

Viability of Transected Follicles

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Follicular trauma during hair transplantation process can take many forms including transection, desiccation and prolonged tissue anoxia. Today there are many reports on hair follicle regeneration after horizontal resection. In the past it has been generally assumed that the germinative center of the hair follicle was exclusively located in the bulbar region. Recently, several Authors have provided evidences that follicular epithelial stem cells should be located in the "bulge" area of hair follicle - the portion of the outer root sheath to which the arrector pili muscle attaches. According to this hypothesis, transversely transection a follicle should allow to obtain 2 viable hair grafts. Unanswered questions emerging from performed in vitro studies are if survival and shaft-production of bisected follicles, as observed in vitro, might be the same when transplanting them in a conventional hair transplantation procedure and what could be long-term behavior of such grafts. The aim of this study was to evaluate in a clinical study survival and growth rates of transversely sectioned human hair follicles.

Ten male with a Norwood Class VI and VII male pattern alopecia were selected for this study. Three templates with diameter 1 centimeter were made by tattooing in middle of bald vertex area. 20 one-hair follicular units were planted in the middle templates. 20 follicles per each patient were transversely transected, parallel to the epidermal surface, immediately below the bulge area in order to obtain 20 lower-half follicles and 20 upper-half follicles. Upper-half follicles were planted in the right templates. 6 and 12 month later the follicular survival was determined by counting terminal hair growth in these recipient sites. 96 - 97% follicular survival was obtained in the middle (control) templates and only 20,5% and 14,5% in the left and right templates.

Key words: follicle, transection, survival, hair transplantation

Modern hair transplantation based on the method of follicle unit transfer allows reaching wonderful cosmetic results when correcting even very apparent androgenic alopecia^{2, 3}.

Basic and actually the only problem limiting the possibilities of hair transplantation is the shortage of donor supplies. Therefore, today

and probably in the future, at least by the period when cloning technologies will be providing transplantologists with unlimited amount of human follicles, main objective of hair transplantation is to save donor supplies to the maximum and increase survivability of grafts to be transplanted. During the excision of the donor's graft and preparation of follicle units from it, as well as during their transplantation certain number of follicles is damaged; irreversibly damaged follicles are subsequently lost. In the recent years new methods of producing and preparation of donor materials allow to better save donor supplies. Rejection of multiple-blade scalpels and the preference of one-bladed ones decreased several times the percentage of transection of produced follicles³. The use of stereomicroscopes with 10-20-fold magnification also reduced the likelihood of the follicle damage during their preparation from donor's graft. According to J. Cooley percentage of follicle transection during microscopic preparation of grafts is reduced twice⁴, while the number of produced hair follicles increases at least by 17% according to studies of R. Bernstein and W. Rassman¹. New methods of producing individual follicle units directly from the donor area, i.e. the procedure of extraction of follicle units also allowed minimizing of transection. Though in spite of sophistication and accurateness of methods of producing donor materials and graft preparation the only consequence is less likelihood of the follicle damage; it is hardly possible to fully exclude transection of all produced follicles.

On the other hand instruments and technologies commonly used in transplantation actually permit high percentage of transection. It means not only producing of donor materials by multiple-blade scalpels, but also using various tools for the automatic preparation of grafts³. The arguments cited in favor of automatic methods of graft preparation are divided into two groups: firstly, follicular trauma during transplantation comprises not only mechanical damage (transections), but also dehydration and anoxia of grafts caused by long period of existence outside human body. E. A. Mangubat considers that automatization of graft preparation increases the risk of transection, but simultaneously decreases dehydration and anoxia of donor's tissue due to considerable reduction of time of the operation. On the other hand transection could not be viewed as irreversible damage to follicles, necessarily resulting in their death. There is strong evidence that cut off parts of follicles are still able to regenerate and produce hair^{5,6,7,9}. According to modern concepts stem cells triggering the development of follicles are located not in a bulb, as it was considered traditionally, but in the area of prominent part of an external root sheath, where erector muscles of hairs connect. According to this hypothesis halves of follicles produced by means of horizontal transection at cervical level could produce 2 viable hair grafts^{5,8}. Studies performed in vitro by E. Raposio et al. showed that survivability of intact follicles (92%) do not differ from the survivability of its upper (91%) and lower (89%) halves placed for 10 days in Williams E. solution and this fact is statistically reliable. The authors emphasize that maximum period for the observation of the culture of human hair is close to 10 days and that clinical research is necessary to study long-term results of bisection of follicles⁹. J.-Ch. Kim и Y.-Ch. Choi⁵, who transplanted fragments of several follicles produced from back of the head to the hip area showed that only upper and lower halves, as well as upper and lower two thirds of the follicles (those parts that included stem follicular epithelial cells in the area where erector muscles of

hair connected) were able to regenerate and produce hair. Regeneration of lower and upper thirds of follicles was not observed⁵. On the basis of these investigations the authors made courageous conclusion that hair transplantologist surgeons might be assured by the fact that transplanted hair can grow despite the considerable damage to a bulb or follicle funnel⁵. On the other hand, J. Cooley demonstrated the effects of delayed growth or its absence when dermal papilla of a follicle was damaged during its extraction, preparation and graft implantation⁴. J.-Ch. Kim informs that regenerated follicles never reach full sizes of intact predecessors⁶.

Therefore, today the issue of survivability of cut off follicles and their steady ability to produce healthy hair is not clear due to unavailability of extensive and reliable clinical research data. Our study aimed to perform series of long term clinical observations in order to get answers on these questions.

Materials and methods. From January to May 2003 selection of volunteers with Norwood VI-VII class androgenic alopecia was performed in our clinic; it was planned to perform first hair transplantation operations on the volunteers on hair line area, forehead and front part of the parietal area. All candidates of the observation should have had absolutely bald areas on the crown of the head where at that stage hair transplantation was not planned. 10 volunteers 35-55 years of age were selected. Donor materials were harvested by single-blade scalpel as elliptical strips. These were dissected under the stereomicroscope. Number of produced follicular units varied from 1880 to 2540. 40 mono-grafts without visible damages were selected from the produced grafts for each specific case. 20 out of them were placed separately on containers (Photo 1) , while follicles of other 20 mono-grafts were exposed to horizontal bisection by the scalpel blade under the stereomicroscope. The follicles were cut off strictly in the middle between dermal papilla and epidermis, i.e. at the connection area of the erector muscle of hairs. Upper and lower halves of the grafts were placed separately (Photo 2-3) .

After outlining of the hair line three 1 cm diameter circles were marked on the top of the head (i.e. square of each circle was equal to 0,785 cm²) (Photo 4) . The recipient site was created by #20 caliber Nokor needles on the hair line, forehead and front parts of the parietal area. On the top of the recipients sites were formed only in the mentioned circles - strictly 20 recipient sites in each of them, i.e. density of placement was \approx 25 grafts/cm². By means of jewelry tweezers recipient sites were filled by follicle units. In the central circles (#1) were implanted 20 intact mono-grafts that were put aside; in the left circles (#2) – 20 upper halves of follicles, while in the right circles (#3) – 20 lower halves (Photo 5) .

The patients were consulted after 1-3 weeks and 3, 6, and 12 months. Six months and a year later after the operation number of hairs were calculated that grew from each of the above mentioned areas restricted by the circles. At the same time neither patients, nor the assistant performing calculation knew how many and what type of grafts were implanted in any of the circles.

Research results and discussion. It should be mentioned that one of the patients left the country; results were received from 9

cases of observation (Table #1) .

The calculation showed that in the central controlled areas (circles) of observation, where intact follicles were implanted, number of grown hair varied from 18 to 20; at the same time almost all hair grew 6 months later ($19.2 \pm 0,12$ hairs on average); for the 12th month an average number of hair was slightly increased ($19,4 \pm 0,05$ hairs on average). Therefore, survivability of intact follicles was equal to 96% and 97% (6 and 12 months later respectively).

In the left and right areas in all cases of observation very delayed and weak growth was mentioned. 6 months later in the left circle where upper halves of follicles were implanted, the growth of hair almost was not observed ($0,3 \pm 0,05$ hairs on average). 12 months later number of grown hair varied strongly from 3 to 8, on average – $4,1 \pm 0,26$. While in the right circle, where lower halves of the follicles were implanted, growth rate was equal to $1,3 \pm 0,25$ hairs 6 months later, and $2,9 \pm 0,28$ hairs 12 months later (Photo 6) .

Therefore, 12 months later after the observation survivability of grafts, representing upper halves of follicles was equal to 20.5%, while survivability of grafts, consisting of lower follicles - 14,5%.

The results of our study proved once more high survivability of grafts consisting of intact follicles. Observed cases of growth, more frequent from the upper halves of cut off horizontally follicles compared to its lower halves (20,5 и 14,3% respectively) also prove the theory that the source of follicle stem cells is not a bulb – the source is the area of cervix of the external root sheath close to the connection of erector muscle of hair. Delayed growth of hair from the cut off parts of follicles is explained by the time necessary for the regeneration of the entire structure of the follicle before it starts to produce hair. Though our clinical observations demonstrated that regeneration of follicles, and therefore, growth of hair in overwhelming majority of cases hair do not take place. From the horizontally cut parts only one out of five upper follicles and one out of seven lower halves survive. Low percentage (20,5 and 14,3%) of survivability prove that while cut off follicles demonstrate ability to regenerate, transection is such a serious trauma that majority of follicles die as a result of switched on mechanisms of apoptosis. We tend to consider transection as the most damaging factor for the follicles that is incomparable with dehydration or increased time of existence outside human body; therefore, today consideration of automatic technologies of graft preparation, as an alternative to manual preparation does not seem to be serious. Moreover, the results convince us that the use of methods for producing and preparation of grafts that allow comparably high percentage of transection, hoping that damaged follicles could still survive and produce hair, is inadmissible. Frequent objections of our colleagues against full microscopic preparation lack scientific evidence and are dictated by economic factors only.

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Cases	Number of hairs grown from:					
	intact follicles		upper halves of follicles		lower halves of follicles	
	6 months later	12 months later	6 months later	12 months later	6 months later	12 months later
1	19	19	0	3	1	2
2	20	20	1	4	0	3
3	19	19	0	3	0	2
4	20	20	0	4	0	3
5	17	18	0	3	1	1
6	20	20	2	8	5	7
7	18	19	0	3	2	2
8	20	20	0	4	1	3
9	20	20	0	5	2	3
M	19.2	19.4	0.3	4.1	1.3	2.9
m	0.12	0.05	0.05	0.26	0.25	0.28
			<i>t= 58.3</i>		<i>t= 57.6</i>	
			<i>p < 0,001</i>		<i>p < 0,001</i>	



Photo 1
20 intact mono-grafts



Photo 2
20 upper halves of mono-grafts transected horizontally in the middle of follicles



Photo 3
20 lower halves of mono-grafts, transected horizontally in the middle of follicles

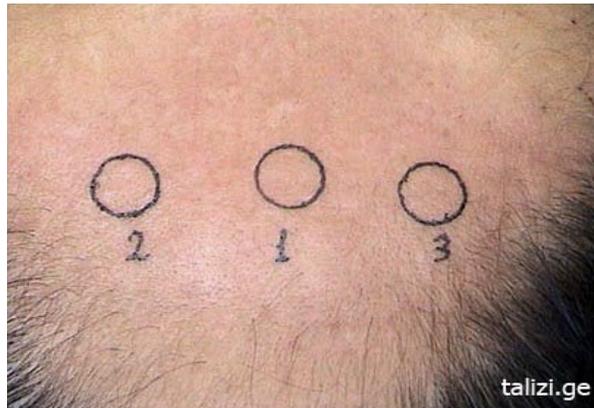


Photo 4
3 circles 1 cm in diameter in the area of hairless crown



Photo 5

20 intact mono-grafts were implanted into the central circle (#1), into the left (#2) circle – 20 upper halves, into the right (#3) – 20 lower halves of follicles transected horizontally at the level of connection of the erector muscle of hair.



Photo 6

Same area one year later