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IN THIS ISSUE

A Histological and Clinical Evaluation of Plasma as a Graft Holding Solution and Its Efficacy in Terms of Hair Growth and Graft Survival

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INTRODUCTION

The surgical trauma of hair transplantation triggers inflammation, which is the first step in wound healing. The brunt of the biochemical changes has to be borne by the newly transferred grafts, which are devoid of any blood supply. Graft survival is affected by a multitude of factors including graft harvesting, dissection, manipulation during implantation, and ischemia/reperfusion injury following implantation in the body. The insults inflicted from these unfavourable factors lead to apoptosis, which affects graft survival and the quality of hair regrowth. In order to achieve the best results, we should focus on improving all of the above mentioned factors.

Grafts harvested and maintained out of the scalp are preserved in a holding solution until they are implanted. Thus, the holding solution plays a crucial role in the hair transplant procedure. An ideal holding solution should have the same osmolality as the graft cells, should prevent acidosis, should provide energy to the cells, and should prevent the release of free radicals. There are two types of holding solutions: extracellular and intracellular. Examples of extracellular solutions are normal saline, Lactated Ringer's (LR), and plasma-like fluids. The intracellular solutions are represented by HypoThermosol®. Extracellular holding solutions do not require chilling, which causes sodium pump failure leading to swelling of the cells, whereas intracellular holding solutions do require chilling.[1]

At our center, we use autologous plasma with platelets as a graft holding solution during hair transplantation surgery. Clinical results have been evaluated with trichoscan analysis and supported by histological evaluation for graft viability. There is literature advocating the use of platelet-rich plasma (PRP) to promote hair growth, based on the logic that platelets have growth factors that stimulate the stem cells of hair follicles.[2]

OBJECTIVE

To evaluate plasma as a graft holding solution in terms of its efficacy in hair growth and hair graft survival.

METHOD

A split-scalp study was carried out comparing grafts transplanted on the right and left fronto-temporal areas selected as recipient sites in the same patient. In the initial study, we used mainly FUT grafts, and in the last phase, we used FUE grafts. A total of 6 patients have been enrolled in the study so far. The left side was designated as the control area (Group A), and the right side behaved as the test area (Group B), for comparison of the results. The right fronto-temporal area received grafts preserved in autologous plasma, while the left fronto-temporal area received grafts preserved in LR solution. Both sides were implanted with grafts harvested using the same technique, with an equal number of grafts of the same quality, and with the same implantation time. Grafts on both sides were implanted by two surgeons sharing similar experience and expertise using optical loupes for magnification (4.5×).

Both the autologous plasma and the LR holding solutions with the grafts were maintained at a temperature of approximately 12 ± 2°Celsius, whereas the room temperature was maintained around 18°Celsius.

➤ PAGE 94

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TABLE OF CONTENTS

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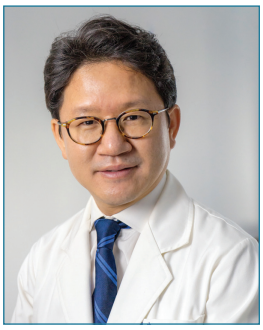

President's Message

Sungjoo (Tommy) Hwang, MD, PhD, FISHRS | *Seoul, South Korea* | president@ishrs.org

Dear Colleagues,

Recently, I have been attending numerous meetings held by members of the Global Council Society. I visited India in February for HairCon and Dubai in early March for the ISHRS World Live Surgery Workshop. Also, I attended the Taiwan Society meeting in late March and went to Beijing in May to attend a joint conference of the AAHRS–China Association of Hair Restoration Surgeons. The Taiwan Society and Chinese Society are planning to apply to be members of the Global Council.

The joint conference held by the AAHRS and the China Association of Hair Restoration Surgeons was especially meaningful and special. At the meeting, there were a total of 400 physicians, including 300 Chinese doctors and 100 Asian and international doctors. It was the second biggest conference after the ISHRS. At this meeting, many doctors shared their ideas and wisdom via the lectures and six surgical procedures were demonstrated. I was very pleased to be able to meet many of my old colleagues from the ISHRS, such as Walter Unger and Richard Shiell, whom I have not been able to see often. Richard Shiell traveled abroad for the first time in many years, since his retirement in 2006, to meet with fellow physicians and to give advice and encouragement to junior doctors. Furthermore, the knowledge shared by Walter Unger, who has 50 years of experience in hair transplantation, was a great help to many participants. I believe that it was an outstanding academic conference, which gave the opportunity to learn from experienced seniors in the field of hair transplantation.

I am aware that many members have submitted abstracts for the Hollywood ISHRS World Congress and you can expect many new and exciting things at the meeting. We have also invited experts on chemotherapy-induced alopecia, which is frequently encountered in clinical practices. I think this topic will be of great help to many of our members. I am deeply grateful to Parsa Mohebi, Program Chair, and the World Congress Committee members who have been working hard to prepare this Congress.

The ISHRS issues the *Hair Transplant Forum International*, or *Forum* for short, once every two months. Over the past 20 years, many research papers and articles on surgical skills have been published. These medical resources are extremely useful for hair transplant physicians. Unfortunately, until now there has been no way to access previously published materials. In order to access old data, you would need a paper copy of the *Forum* or a pdf file. To tackle this, we are developing an e-publishing platform to enable search functions. When this is in place, you will easily be able to look up research materials published in the past, which will be of great help to our members. I would like to thank Bob True for all his work and efforts on putting this in place.

Regarding potential venues for the World Congress, we have already decided Hollywood, USA in 2018, Bangkok, Thailand, in 2019 and Panama City, Panama, in 2020. We are currently in the process of thinking about potential venues for 2021. Members are welcome to make suitable suggestions, as it would be of great help. In addition, I would like to encourage you to apply for ISHRS Research Grants and recommend candidates for the Platinum Follicle Award, the Golden Follicle Award, the Distinguished Surgical Assistant Award, and the Board of Governors. I am sure that there are many suitable candidates for these grants, awards, and positions.

Lastly, I would like to sincerely thank you all for your effort and continuous support for the development of the society. I will do my best to assist in any way. ■

Co-editors' Messages

Andreas M. Finner, MD, FISHRS
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I just returned from the Meeting of the European Hair Research Society in Bologna (www.ehrs.org). A lot of research was presented.

The hair follicle is an exciting object to study for basic scientists. This includes hair immunology, stem cells, signals, receptors, cycling, inflammation, pigmentation, hair care, and genetics. While male pattern hair loss can be attributed to several genes, female pattern hair loss appears to have a different and poorly understood genetic etiology. Minoxidil works in both conditions but some patients have a deficiency of the activating enzyme sulfonyl transferase and need higher concentrations.

As explained in this issue's Literature Review, topical finasteride may become a new treatment option. However, in higher doses, it also suppresses systemic DHT, which would diminish the potential advantage over oral finasteride. PRP is increasingly used, but platelet lysate produced with ultrasonic waves may contain even higher concentrations of growth factors.

Hair transplantation lectures and sessions have become a regular part of hair research meetings. This is because more and more studies have demonstrated the effect of hair surgery to improve advanced alopecia.

This issue contains a study on using plasma as a holding solution and a Cyberchat discussion about graft chilling. More and more data on the final outcome of different techniques and instruments will further improve the acceptance of our work and results.

To have your report be published in an upcoming issue of the *Forum*, please email it to forumeditors@ishrs.org. ■

Bradley R. Wolf, MD, FISHRS
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To be successful in our field, surgeons need stay current on the increasing number of topics and details necessary for successful and maximal hair regrowth. In this issue, we are fortunate to present articles on holding solutions, fat cells to stimulate follicles, and topical finasteride, among other useful and pertinent topics.

I have heard Anil Kumar Garg's lecture on plasma with PRP as a graft holding solution at more than one meeting and have been impressed with his results, which we present here. Although obtaining and using plasma and PRP is more difficult than other holding solutions, the impressive results suggest it may be worth the extra effort, but further studies are certainly warranted to prove this.

Robin Unger's Cyberspace Chat presents important contemporary and practical information on holding solutions from some of our more experienced surgeons as well as the results of studies performed on holding solutions. I urge everyone who uses holding solutions to read this column.

We welcome Mario Marzola's first Editor Emeritus column. Mario and fellow past Co-editor Bob True were certainly mentors for me and I admire their ability to remain at the forefront of new developments in our field. Mario writes about using fat cells to unlock the potential of miniaturized follicles while Bob is hard at work updating our website to improve the *Forum* search function as well as making the articles more visible to the medical community.

As the lay press and literature continue to vilify finasteride, topical finasteride is getting more interest by those concerned about the potential side effects of finasteride. In Literature Review, Nicole Rogers examines an article which summarizes the results of seven studies regarding the efficacy of human *in vivo* topical finasteride treatment. In general the studies appear to support the use of topical finasteride.

From 2008–2011, the Hair or Basic Sciences column was written by Nilofer Farjo. Jerry Cooley took over from 2014–2016. We would like to thank Nilofer and Jerry for their work on this column over the years. Jerry has decided to turn the reins over to Vladimir Ratushny who practices in Beverly, Massachusetts, about 30 miles northeast of Boston. Dr. Ratushny grew up on Long Island, New York, completed a combined MD–PhD Program at Drexel University College of Medicine and Fox Chase Cancer Center in Philadelphia, and completed his dermatology residency training at the Harvard Dermatology Residency Training Program. We thank him for accepting this position and look forward to his learned contributions.

We would also like to thank Bob Niedbalski, President of the ABHRS, for his update in addition to all columnists and article authors for their contributions. ■

Notes from the Editor Emeritus, 2014–16

Mario Marzola, MBBS | *Adelaide, South Australia* | mario@marzola.net

Hello, friends and colleagues! This is my first Editor Emeritus column. How time flies! I have always enjoyed reading the *Forum*s, no more so than now. Congratulations to Brad Wolf, Andreas Finner, and all the columnists. The journal has never been in better hands.

My world continues to be dominated by the unsolved mysteries of medicine, none more than the mysteries of hair biology. Rather than follicular neogenesis in a laboratory, then planting the new follicles into the scalp, the Holy Grail for me is the reversal of miniaturization of our existing follicles. See the photos in Figure 1, the second of which is computer generated as you would have guessed. But why not dream of this? We already know that hair can move from vellus to terminal and back to vellus again, and what's more, make that transition over and over. All that is needed are the appropriate signals.

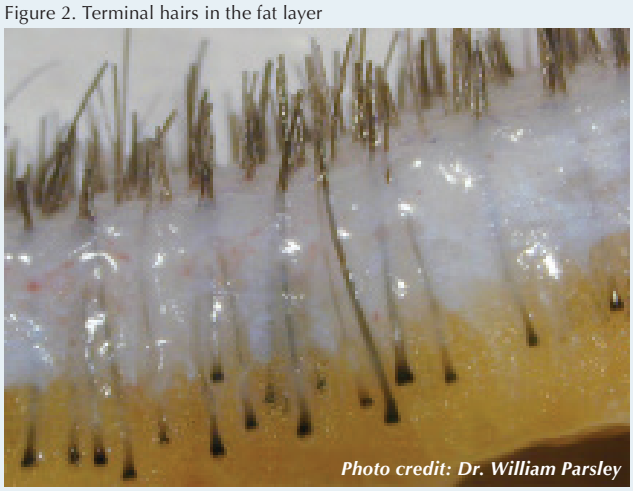
Figure 1.

We also know that the miniaturized follicles can live in the vellus state for many years. Some say forever, but most researchers would agree that there is an attrition rate sooner or later. Nevertheless, every person currently miniaturizing would still have all the follicles alive and capable of reversal if we knew the way. Today, we are already seeing reversal of miniaturization with finasteride and dutasteride. I'm sure we have all been amazed when the occasional patient returns with a "wow" factor of much more hair growth than before. I'm seeing it occasionally with these drugs but more with oral minoxidil. Even more with the two combined. How can we increase the number of these "wow" factor patients?

With our current medications, the untapped potential is most likely in the potassium channel openers/activators like minoxidil. There are many other medications with the same action so the possibility of improving the effect on hair is enticing. It was Professor Valerie Randall of University of Bradford who shed light on the potassium channels, but I believe she has retired. Now this may be a great opportunity for a PhD student to take it further. By good fortune or by good research, we will find the answer.

Another fascinating area that is emerging is the relationship between anagen follicles and fat cells. Big, healthy terminal hairs bury their bulbs well into the fat layer (Figure 2, courtesy of Dr. William Parsley). Bald scalps possess much less fat than scalps with a full head of healthy hair. It seems that we can't have one without the other. Another Valerie, Dr. Valerie Horsley of Yale School of Medicine, speaks of a correlation as yet unexplained between fat and hair growth. Even before we can discover why terminal bulbs need fat cells, why don't we give scalps with miniaturizing hairs a fat transfer? Many case studies and trials doing just that are on their way; let's watch this space.

Figure 2. Terminal hairs in the fat layer



Fat, as we know, contains a large reservoir of stem cells, which some of us have used successfully in the treatment of osteoarthritis. In this space, the fat is processed to remove the large lipid-filled cells leaving behind a "soup" called stromal vascular fraction (SVF). This contains stem cells, progenitor cells, pericytes, endothelial cells, fibroblasts, and some red and white cells as well as extracellular matrix and damaged cells. SVF has been successful in treating osteoarthritis but not so in hair loss. Many of us have injected SVF into balding scalps with little to no benefit. Is it possible, therefore, that the secret ingredient for hair growth is in the fat cell itself?

History will tell us that nothing is forever, especially in this fast-evolving field of hair restoration. Less invasive treatments have been the feature of the evolution of hair restoration in my 40-year tenure, perhaps soon we will have good enough reversal of miniaturization to dispense with surgery. Burns, accidents, and scarring alopecia loss I imagine will still need surgery, but hopefully for our average MPHL and FPHL patients… no. ■

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The following parameters were taken into consideration for the study:

- A histological study with MTT stain (a colorimetric assay for assessing cell metabolic activity) was done in order to confirm the viability of cells in the grafts at 12 hours and 72 hours.
- Periodical post-operative patient follow-up with regular photographs and trichoscan evaluations was used to identify any event of anagen effluvium due to post-surgical shock loss.
- Trichoscan study for hair density was done at 3 months for hair growth.
- Hair thickness was assessed at 6 months and 12 months for the quality of hair growth.

Preparation of autologous plasma

Preparation of autologous plasma was the first step before performing the hair transplant. We collected 23cc of blood from the patient in a syringe with 2cc ACD (acid citrate dextrose) solution as an anti-coagulant. The blood was transferred to a high-quality glass container designed by the author. The blood was centrifuged in a temperature-controlled (19°Celsius) centrifuge machine at 5,000 RPM (rotations per minute) for 16 minutes. The process resulted in the separation of red blood cells (RBCs) at the bottom of the tube and plasma with platelets forming the upper fluid compartment. The 23cc of blood yielded approximately 12cc of plasma. Hence, we can deduce that the platelet concentration was twice normal levels. The lab further confirmed that the platelet count ranged between 400,000–500,000/mm^3. The plasma created was then stored in a sterile stainless steel bowl (a petri dish can also be used) maintaining a temperature of 12° ± 2°Celsius on a cool gel pack ready to receive the grafts.

The harvested grafts were divided randomly into two groups, with an equal number of grafts per side. Control Group A grafts were stored in LR solution and test Group B were stored in plasma solution. The temperature of both graft holding solutions was maintained as the same. Grafts dipped in plasma formed a very loose clump. A trained assistant separated the individual grafts from a small clump and placed it on the surgeon's hand to implant.

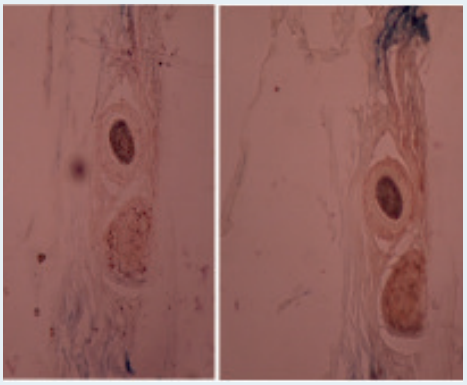
Six volunteer patients ranging in age between 25 to 40 years old and having similar grades of male pattern hair loss (MPHL) were included in the study. Grafts were implanted over the bilateral fronto-temporal areas as planned. Grafts stored in plasma solution were implanted on the right fronto-temporal side and the grafts stored in LR solution were implanted on the left fronto-temporal side. The same number of grafts were implanted resulting in a standard density of 40 grafts/cm^2. Routine post-operative care of the donor and the recipient areas was followed as per general guidelines for all hair transplant patients. The study was conducted as follows:

- The graft samples A and B were sent for MTT staining at 12 hours and 72 hours of graft holding time in order to determine viability of the cells.
- Patient follow-ups were conducted at 1, 2, 3, 4, 6, and 12 months after transplant.
- Photographs were taken for comparison of the left and right fronto-temporal areas with and without flash.
- Hair count and density were taken on both sides using trichoscan.
- At the 6- and 12-month follow-ups, photographs and trichoscan for hair thickness were repeated for evaluation of terminal hair.

OBSERVATION

MTT staining at 12 hours showed that grafts stored in LR solution showed poor staining, while the grafts stored in plasma solution were well stained, indicating good cell viability in the plasma group when compared to the LR group (Figure 1). MTT staining at 72 hours showed grafts stored in plasma showed good staining while the LR group showed very poor staining.

FIGURE 1. MTT staining at 12 hours



Trichoscan study

As shown in Table 1, the hair count and density in the plasma group were significantly higher than in the LR group. The unpaired t-test showed the LR group had a mean 4.5 ± 2.95 SD and the plasma group had mean 27.50 ± 4.135 SD with P-value < .001.

TABLE 1. Hair Count and Density at 3 Months on LR Side and Plasma Side
(Implanted density: 40 grafts/cm^2)

	Hair Count		Hair Density (per square cm)		Average % of Hair Growth	
G.H. SOLUTION	RL	PLASMA	RL	PLASMA	RL	PLASMA
	2	9	6.6	29.7	11.00%	68.75%
	1	10	3.3	33		
	2	7	6.6	23.1		
	0	8	0.0	26.4		
	2	7	6.6	23.1		
	1	9	3.3	29.7		
AVERAGE	1.3	8.3	4.4	27.5		

CLINICAL EVALUATION

Photographs of the right and left fronto-temporal areas were taken at 50 days (Figure 3) and 120 days (Figure 4) after hair transplant for evaluation of hair growth and anagen effluvium. Also shown is a patient 120 days after transplant. Plasma holding solution was used for all grafts (Figure 5).

DISCUSSION

The most important benefit of a graft holding solution would be an increase in hair yield from the transplanted grafts. The optimum holding solution would reduce the damage from reperfusion injury and free radical formation as well as from ionic imbalance and variation in osmolality created by the ischemic phase.

Holding solutions are formulated according to the composition of intracellular and extracellular body fluid environment

FIGURE 3. Immediate post-op *(top)* and 50 days post-op: plasma *(bottom left)*, Lactated Ringer's *(bottom right)*

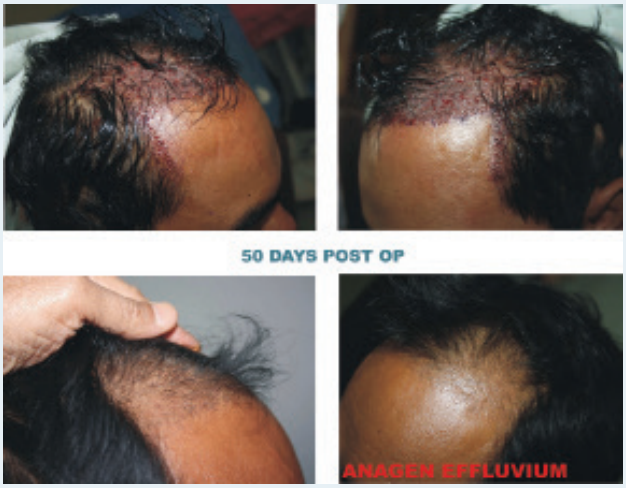


FIGURE 4. Pre-op *(top)* and 4 months post-op: patient's right hairline *(bottom left)*—plasma, left hairline—LR; LR side (*middle photo*); plasma side (right photo)

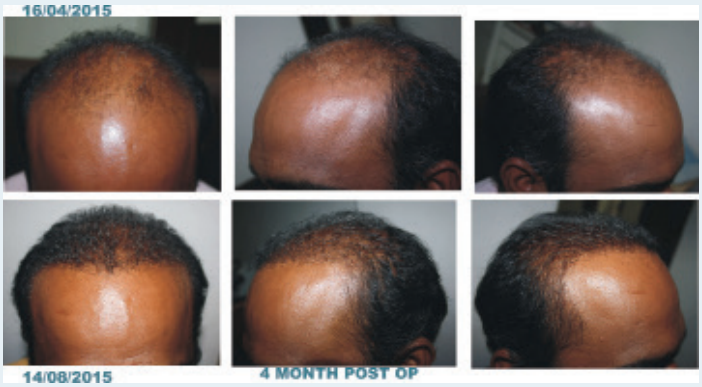
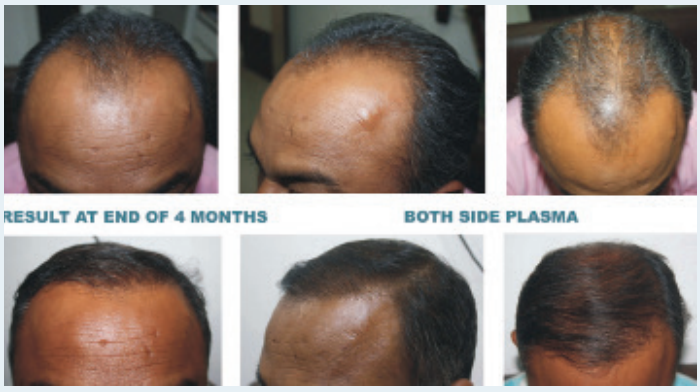


FIGURE 5. Pre-op *(top)* and 4 months post-op *(bottom)*; both sides plasma holding solution



and behave differently. An intracellular graft holding solution needs chilling, which is not user-friendly.[1] It also does not ensure protection from reperfusion injury and is expensive.[3] Extracellular solutions are widely used, economical, and do not need chilling. Intracellular fluids like hypothermasol with ATP added have significant benefits when graft holding time is more than 10 hours, however, this is a very rare situation as most hair transplant procedures are complete within 4-6 hours.

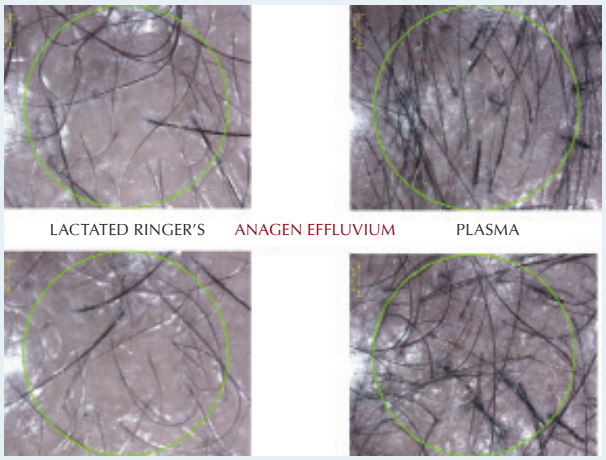
Autologous plasma is an extracellular fluid that is isotonic with nutrients and platelet-derived growth factors. It is cost effective and can be prepared by a surgeon or a pathologist. Drying and dessication of grafts immersed in plasma is delayed, and grafts look shiny and more hydrated even at the end of 4 hours holding time. Ubel in his study had implanted grafts after dipping in plasma and reported a 5 to 53% increase in hair count after 7 months of hair transplant.[2,6]

MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) Assay

The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes reflect the number of viable cells present under defined conditions. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethyl-thiazole-2-yl-2,5-diphenyl tetrazolium bromide) to its insoluble form.[9] Therefore, the dye can detect metabolically active live cells. In our study, samples of hair follicle grafts were sent for MTT histological assay in order to detect live cells. Results of staining showed that at 12 hours the grafts held in plasma solution were better stained than those held in LR solution. Staining results at 72 hours were surprising: plasma grafts showed good staining while LR grafts showed very poor staining indicating that the cells were viable in plasma grafts even at the end of 72 hours.

Trichoscan study done at 3 months for the hair count on the LR side showed an average of 1.3 (density 4.4g/cm^2); on the plasma side, the average hair count was 8.3 (density 27.5g/cm^2). Thus, the plasma side had 68.75% hair growth, while the LR side had only 11% growth. The unpaired t-test showed mean 4.5 ± 2.95 SD for the LR side and mean 27.50 ± 4.135 SD for the plasma side with a P-value < .001, which is significant. This indicates that anagen effluvium (Figure 6) on the plasma side was 31.25% while on LR side the effluvium was 89.00%. This shows that anagen effluvium was controlled by 58.75%, which is significant (P-value < .001).

FIGURE 6. Photomicrograph 50 days post-transplant showing significant anagen effluvium on LR side *(left)* while on plasma side *(right)* implanted hairs were still present.



In the first 7 days following a hair transplant, there is a period of inflammatory response (involving neutrophils, eosinophils, macrophages, platelets, fibroblasts and growth factors[2]) in which both erythema and edema occur followed by apoptosis and the grafted, as well the existing, hair follicles may enter into an involution phase resulting in hair shedding. This process is triggered and propogated due to ischemia. The follicles become refractory and those that survive will regrow at the stimulus of

➤ PAGE 96

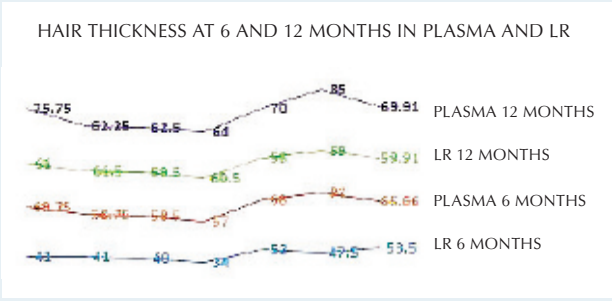
CONTINUED FROM PAGE 95

the next growth cycle, which begins after the third month and continues up to the seventh month. Prevention of anagen effluvium can be achieved with prevention of apoptosis of the more metabolically active progeny of the stem cells. This observation may help us in the development of an ideal holding solution by further bio-enhancement of platelet and plasma solution.

Hair thickness measured at 6 months by trichoscan showed an average of 53.5 µmm on the LR side while on plasma side it was 65.66 µmm, which was significantly higher (Figure 7). The unpaired t-test showed the LR group mean 53.5 ± 6.377 SD and the plasma group mean 65.67 ± 9.688 SD. The P-value was <.001, which is very significant.

At the 12-month follow-up, hair thickness measured 60.6 µmm on the LR side while on the plasma side it was 66.125 µmm. The thickness of hair on the LR side increased by 12 months but was still less than on the plasma side. The unpaired t-test showed the LR group mean 60.00 ± 1.414 SD, and the plasma group mean 69.86 ± 9.218 SD. The P-value was < .001, which is very significant.

FIGURE 7. Hair thickness in both groups at 6 months and 12 months was compared. There was an improvement in hair thickness in both groups from 6 months to 12 months with P-value .002.



Hair diameter depends on a number of viable cells in the matrix. These are the mesodermal stem cells known to be very sensitive to ischemia. Ischemia leads to accumulation of free radicals and anaerobic metabolic pathways resulting in apoptosis of cells thereby affecting hair thickness. The hair thickness on the plasma graft side was better than on the LR side. This may be because of the effect of multiple beneficial factors in the plasma holding solution.

Platelets are activated on contact with collagen around hair follicles resulting in the release of various platelet derived growth factors.[15,16] Fibrinogen in plasma gets converted to fibrin, which forms a mesh in which platelets are trapped.[2,4-6] This fibrin mesh with activated platelets forms a 3D fibrin scaffold. Platelet-rich fibrin (PRF) was first described by Choukroun et al. in France.[10,11] Fibrin glue along with skeletal myoblasts in the fibrin scaffold preserve cardiac function after myocardial infarction.[12] *In vitro* prefabrication of human cartilage is created in shapes using fibrin glue and human chondrocytes.[13] Long-term regeneration of human epidermis is achieved on third-degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix.[13] There is a definite role of the fibrin matrix in angiogenesis.[14]

CONCLUSION

Autologous plasma is an easily available graft holding solution. It is isotonic in nature having nutrient growth factors as well as the advantage of fibrin. Platelets along with the plasma provide multiple growth factors promoting epithelialization and neovascularization, and action on hair follicle stem cells to improve growth. The fibrin coating around the graft makes it sticky and prevents dehydration. The growth factors and nutrients successfully prevent the anagen effluvium and shock loss post hair transplant. The thickness of hair and yield of the graft is also better in plasma. The split-scalp controlled study certainly shows the advantages of using plasma over other extracellular graft holding solutions. At the same time, it is not an ideal graft holding solution where chilling cannot be done and the availability of energy source is not clear. But this can be developed as an ideal graft holding solution by some innovative bioenhancement.

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