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Concepts and Challenges in Hair Follicle Cloning

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Introduction

It was shortly after World War II that Lille and Wang first demonstrated that feather follicle development is dependent on mesenchymal-epithelial interactions.¹ Mechanisms underlying follicle development are reprised in the development of other appendages, and so these observations by Lille and Wang paved the way for advances in the field, in particular regarding the recognition that all hair follicle development and adult activities are regulated by interactions between the mesenchyme and the epithelium. Later on, Oliver was the first person to demonstrate that rodent mesenchyme-derived papilla, when isolated from the follicle, can initiate these interactions and induce new hair follicle growth in adult skin.² Since this, a multitude of experiments have demonstrated that both intact papilla and also cultured papilla cells are capable of inducing de novo hair growth not only in skin, but several other types of epithelia.³ Interestingly, one other striking behaviour of cultured rodent whisker papilla is their propensity to aggregate, both *in vitro* and after subdermal injection.⁴ Cultured rat dermal papilla cells are capable of self-aggregating to form condensate-like clumps, while we have never observed this aggregation phenomenon after injection of human cells into the skin.

Dichotomy of Activity Between Hair Follicle Dermis and Interfollicular Skin

We have previously proposed that dermal papilla, sheath, and fibroblasts are not in a steady state within the skin.⁵ Moreover, there is experimental evidence supporting the lack of a steady state between the papilla and sheath cells during the follicle cycle.⁶ We believe that hair follicle dermal cells may have an additional role in skin, acting as wound healing fibroblasts in the context of skin injury or trauma.⁷ This idea is supported largely by the observation that hair follicle dermal cells assume different roles after cell culture. Once in culture, hair follicle dermal cells can act as mesenchymal stem cells and differentiate down a variety of mesenchymal lineages.⁸ This raises the question of whether cultured hair follicle dermal cells will act as hair follicle cells, or in another capacity, when transplanted back into the skin for the purpose of hair follicle regeneration.

Strategies for Targeting Follicle Regeneration

For several years, researchers have been trying to exploit the inductive potential of the dermal papilla and demonstrate that human dermal papilla cells hold the same inductive properties as rodent cells.⁹ To this effect, there are currently two experimental strategies that utilise hair-associated dermal cells for follicle regeneration. The first of these involves injecting cultured dermal cells into the dermis, where it is hypothesized that they will augment existing follicles, and transform a vellus follicle to a terminal fate by contributing to, and enlarging the size of the dermal papilla. This is supported by observations that the size of the dermal papilla is directly related to the size of the hair fibre produced.¹⁰ The second strategy involves injecting or grafting hair follicle dermal cells so they are in contact with skin epithelium, where it is proposed they will initiate mesenchymal-epithelial interactions to instruct new follicle growth. By and large, these experiments have been unsuccessful, and to understand this, we have to go back and look at the behaviour of human hair follicle dermal cells when compared to their rodent counterparts (Figure 1). In the absence of spontaneous aggregation by human papilla cells, they may be behaving as fibroblasts in what is essentially a wound environment after their injection or grafting. Coupled with their loss of specificity by culture, hair follicle dermal cells will not necessarily incorporate into a hair follicle, but rather will contribute to the surrounding interfollicular tissue.

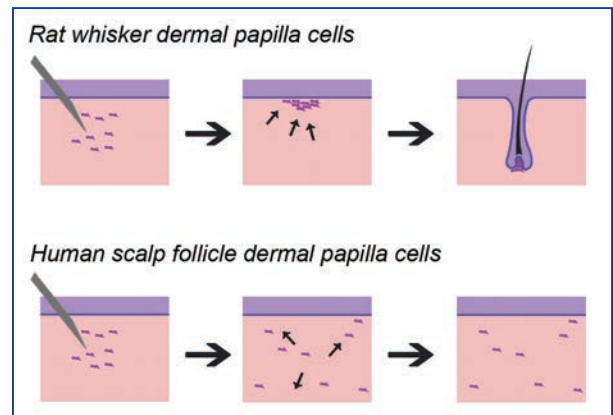


Figure 1. Cartoon illustrating differences between rat and human dermal papilla cells. After injection into skin dermis, cultured rat whisker dermal papilla cells have a propensity to aggregate, while human cells often act in an opposite manner and disperse. The aggregation of rat dermal papilla cells enables them to initiate epithelial-mesenchymal interactions, resulting in the growth of de novo follicles in specific sites.

SAVE THE DATE!



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President's Message

Vincenzo Gambino, MD *Milan, Italy*

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I am planning as much as possible to devote each of my messages in the *Forum* to an issue that is important to a different nation or region. While we are universally involved with the subject of hair restoration and science, we face different situations because of what is and is not allowed where we practice medicine.

In this issue I want to talk about a very big problem in the United States that may "infect" other parts of the world.

Currently, the Board of Governors of the ISHRS is receiving more and more letters from concerned doctors regarding the proliferation of tech-organized hair restoration practices that hire doctors untrained in hair restoration to supervise and legitimize the office.

Why is this happening now?

Many doctors in other fields of medicine are seeing their income shrinking as government and insurance companies are reducing fees for services and they are drowning in paperwork to receive payment.

Esthetic medicine is their solution. Some manufacturers of FUE medical devices are seeing a big financial opportunity. They are marketing a "turnkey" model to these doctors. They supply the equipment and the trained techs who perform the surgery. The doctor in effect becomes the "front man." Now we see this going one step further—techs themselves are marketing that they can run a hair restoration practice for you under your medical license.

With the increased interest and demand for FUE, there is no strip removal, and in some jurisdictions, licensed non-physician ancillary staff is legally allowed to harvest and place under the supervision of a doctor. The patient's assumption being that the supervising doctor specializes in hair restoration.

Why is this dangerous?

Hair restoration surgery is not a one-size-fits-all proposition. There is the medical diagnosis, current classification, prediction of future hair loss based on age, quality of existing hair, family history, donor availability, anesthetic concerns, medication and medical treatments, and many other possible factors. We have all seen bad hair restoration, but I am afraid that patients can face much worse consequences in this shady scenario.

What worries me most is there seems to be no easy solution to this problem that already was predicted by past presidents. The Board of the ISHRS is monitoring the situation and looking at possible avenues to address the issue, but the Society is much larger than just the Board. I would like to tap into this larger pool of minds and ask any of you with an idea to contact me. I will respect your confidentiality. ♦

Co-editors' Messages

Mario Marzola, MBBS Adelaide, South Australia editors@ISHRS.org

Hello! Welcome to every hair transplant surgeon in the world. Whichever country you live in, whatever your background training, if you are interested in hair restoration, you are welcome here at the ISHRS. Please join us in sharing all aspects of hair restoration—surgical and medical—so we can learn from each other for the benefit of our patients.

We are always interested in North and South America and Europe where a lot of experience lies, but, increasingly now, we are interested in the East. Middle East, Far East, and South-East Asia, where three-quarters of the world's population lives.

Within the ISHRS are doctors with great experience and great knowledge of the hair follicle who are very keen to teach and share that knowledge. My co-editor and friend, Dr. Bob True, will confirm that articles for this *Forum* from new doctors are greatly sought after. So please put something together and send it to us. When you share your thoughts, concerns, or good outcomes other doctors are able to learn from your contributions as well as provide feedback or additional insight. Also, at our meetings, the "same old faces" would like nothing more than the program chair to announce that new presenters will take their place. If you need it, there is plenty of assistance available to help you take the step to publishing and presenting.

What's New? Bob and I have the pleasure of guiding this *Forum* for the next three years. Just to show our commitment to the "International" word in our name, we will aim to publish international contributions as often as possible. If the English language is a concern, seek the help of a translator and try to edit it as well as you can. We will do the rest once you send it in.

You may be aware that the ISHRS has a Global Council meeting at every annual conference where each country's hair society meet to discuss what is happening around the world. Presently, there are 17 such societies. Bob and I hope to feature a society in every issue of the *Forum* during our tenure. In this issue, we have an interview with Prof. Franco Buttafarro, President of the Italian Society of Hair Restoration (ISHR). If you do not have a hair society in your country, let's start one up immediately, we know how.

All the leaders in the ISHRS believe that once we have knowledge it is our responsibility to teach and share that knowledge with all who seek it. This is our take-home message in our first edition as editors. We believe it will be the same message in three years' time. Let's enjoy our time together. ♦



Robert H. True, MD, MPH New York, New York, USA editors@ISHRS.org

I am excited about joining Mario Marzola as the new *Forum* editors. I am excited because I believe we belong to a dynamic international specialty society full of the spirit of innovation and collaboration. I expect to have a lot of worthwhile material to publish over the next three years.

Drs. Nilofer Farjo and William Reed did an outstanding job as co-editors. They brought informed, precise, inquisitive intellects to the journal. It is a bit daunting to follow them, but at the same time I am very grateful for their contributions and giving us great role models to emulate.

I am very pleased to welcome a great lineup of columnists. Dr. Jerry Cooley will report on *Hair Sciences*; Drs. John Cole and Bradley Wolf will share the *Cyberchat* column; Drs. Nicole Rogers and Jeffrey Donovan will cover *Review of the Literature and Studies*; Dr. Tim Carman will take over the *How I Do It* section; Dr. Sara Wasserbauer will continue her entertaining and informative *Hairs the Question*; Dr. Marco Barusco is launching a new column, *Difficult Cases and Complications*; Dr. Russell Knudsen will continue *Controversies*; and one of my personal favorite features, *Editor Emeritus* will continue, starting with the always informative Dr. Francisco Jimenez in this issue.

The San Francisco meeting was very successful based on reviews and comments. That success was made possible by the great location along with a really solid scientific program. The report on the meeting in this issue recaps much of the meeting general sessions. But the reporting does not cover the workshops, symposia, and expert tables. As Program Chair, I had the opportunity to wander around and see almost all of them, and I thought the faculties did a wonderful job making these perhaps some of the best meeting content. In particular, I was very impressed with the quality of the Assistant's Workshop. One of the surprise hits

of the meeting was the ISHRS & ABHRS Morbidity and Mortality Review. Many of those who participated said it was the highlight of the meeting for them. Participants found the intimate and frank discussion of significant complications was of superb educational value. I agree and I am pleased that this program will continue at next year's meeting.

Problem cases and complications present learning opportunities. In this issue, Dr. Sezgin's article presents a previously unidentified complication of FUE, and Dr. Barusco describes a masterful management of a very unusual case. I hope for more such cases to be part of every issue.

Dr. Colin Jahoda's Norwood lecture in San Francisco received rave reviews, so I am pleased we are able to present *Concepts and Challenges in Hair Follicle Cloning* by Dr. Clair Higgins and Dr. Jahoda as this issue's lead article. ♦



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Vision: To establish the ISHRS as the leading unbiased authority in hair restoration surgery.

Mission: To achieve excellence in patient outcomes by promoting member education, international collegiality, research, ethics, and public awareness.

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- Articles should be written with the intent of sharing scientific information with the purpose of progressing the art and science of hair restoration and benefiting patient outcomes.
- If results are presented, the medical regimen or surgical techniques that were used to obtain the results should be disclosed in detail.
- Articles submitted with the sole purpose of promotion or marketing will not be accepted.
- Authors should acknowledge all funding sources that supported their work as well as any relevant corporate affiliation.
- Trademarked names should not be used to refer to devices or techniques, when possible.
- Although we encourage submission of articles that may only contain the author's opinion for the purpose of stimulating thought, the editors may present such articles to colleagues who are experts in the particular area in question, for the purpose of obtaining rebuttal opinions to be published alongside the original article. Occasionally, a manuscript might be sent to an external reviewer, who will judge the manuscript in a blinded fashion to make recommendations about its acceptance, further revision, or rejection.
- Once the manuscript is accepted, it will be published as soon as possible, depending on space availability.
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- A completed Author Authorization and Release form—sent as a Word document (not a fax)—must accompany your submission. The form can be obtained in the Members Only section of the Society website at www.ishrs.org.
- All photos and figures referred to in your article should be sent as separate attachments in JPEG or TIFF format. Be sure to attach your files to the email. Do NOT embed your files in the email or in the document itself (other than to show placement within the article).
- We CANNOT accept photos taken on cell phones.
- Please include a contact email address to be published with your article.

Submission deadlines:

February 5 for March/April 2014 issue

April 5 for May/June 2014 issue

A Note from Dr. Mario Marzola:

The Learning Never Stops—
201 Years of Experience in One Room

On November 22, 2013, with several of my colleagues, I found myself learning in the operating room of Dr. Russell Knudsen in Sydney, Australia. He was demonstrating a female hairline lowering operation using follicular unit transplants. Besides being too high, the patient's hairline was also a little see-through, not having a strong front edge. For this reason, the alternative way of lowering the hairline with an anterior scalp reduction was not suitable. We watched the harvesting, the making of the sites, the preparation of the grafts, and graft placement, all elegantly done with ease and comfort.

During the session, someone remarked that there was a lot of experience here. Adding it all up, it came to 201 years in



Back (L to R): Drs. Russell Knudsen, Mario Marzola, Bessam Farjo, Vincenzo Gambino and Robert Haber. Front (L to R): Drs. Nilofer Farjo and Richard Shiell.

total—helped by a few “seniors” present including yours truly. Dr. Gambino, our current President was there, also a smattering of past presidents, past *Forum* editors, textbook authors, and award winners. Most enjoyable, thank you Russell.

Notes from the Editor Emeritus

Francisco Jimenez, MD Canary Islands, Spain jimenezeditor@clinicadelpelo.com



Platelet Rich Plasma (PRP) in Patients with Androgenetic Alopecia (AGA): Does It Work?

Introduction

There are numerous doctors and hair clinics worldwide that regard PRP simply as a hip, easy to perform, and, above all, lucrative form of therapy that may or may not work, but at least does no harm. In addition, its application in hair loss disorders is becoming very popular among the general population and many patients are asking for it in our clinics. To illustrate this, a standard internet search for “PRP and hair loss” will give over 3 million hits. However, a similar search in the PubMed scientific literature will reveal a total of just 9 published papers on the subject.

At the 2013 ISHRS Annual Scientific Meeting in San Francisco, I was invited to organize a round table session on the use of PRP in hair loss. Since this is a controversial topic, I invited along a number of highly respected colleagues (including Drs. John Cole, Joe Greco, Bob Niedbalski, Bob Reese, David Perez-Meza, Fabio Rinaldi, and Ryan Welter), who are well known for their experience in the use of PRP. Prior to the meeting, I sent them all a questionnaire addressing a number of key questions such as PRP preparation, injection technique, patient satisfaction, etc. In the absence of evidence-based data, we need to rely on the experience of “PRP experts,” and so I would like to summarize the results of the questionnaires that were returned to me about this complex and controversial subject.

Points of agreement

There are several points in which there is general agreement:

1. *The ideal candidates for PRP:* All experts responded that patients with thinning but not fully bald areas are the best candidates, which includes patients in early stages of AGA and female androgenetic alopecia (FAGA). Patients with AGA Norwood types I-IV and FAGA Ludwig types I-II are better candidates than Norwood types V-VI and Ludwig III.
2. *Assessment of patient satisfaction after PRP injections:* Most experts agree that approximately 70-90% of patients will see some degree of improvement (this is a subjective assessment since no randomized clinical trials have been performed using objective measurements of hair mass/density). Around 20% will be disappointed with the results. However, when questioned about the realistic outcomes that the patients are told can be achieved with PRP, most of the experts keep patient expectations relatively low, stating that they expect a modest improvement in the diameter of miniaturized hair and the maintenance of existing hair.
3. *Time when improvement in hair growth is expected to be seen:* Most of the experts were of the opinion that improvement would be seen between 2 to 6 months after the PRP injection. Dr. Greco thinks it is important to explain to the patient that the peak effect is at 4-6 months and that the treatment must be continued to achieve long lasting results.
4. *Anesthesia prior to PRP injections:* All use an anesthetic prior to injection, normally ring block with 1% lidocaine.

Differences in approaches

There were several differences in approaches:

1. *PRP preparation:*
 - Joe Greco uses the Emcyte Pure PRP kit.
 - Bob Reese uses the Cytomedix kit.
 - John Cole uses the Angel system.
 - Ryan Welter and David Perez use the Harvest system.
 - Bob Niedbalski uses PRP plus ACell. For the PRP, he uses the Harvest system.
 - Fabio Rinaldi does not use any kit, instead he buys the components separately.
2. *Activation of platelets:* We know that platelets need to be activated in order to release growth factors, but we do not know whether an exogenous activator is needed or, if this is the case, which one works best. Platelets can be activated by exogenous activators (thrombin, calcium, mechanical trauma) or by a natural activator (collagen). In theory, exogenous activation is not needed for soft tissue injections. Some experts use thrombin (Greco, Cole, Perez, and Reese) or calcium gluconate (Rinaldi) or mechanical trauma by multiple injections (Niedbalski and Welter) to activate the platelets. Greco also “stimulates” the scalp with a roller prior to injection.
3. *The number of and interval between PRP sessions required for improvement:* Although in this respect the approach of each expert is different, the majority favor two or more sessions 3 to 9 months apart.
4. *Duration of the increase in hair growth after PRP injection:* Nobody seems to know for certain, but it would appear that the treatment must be continued to achieve long lasting results.
5. *Cases in which PRP is offered to patients:* This seems to be a personal choice with a different approach used by each doctor. Dr. Greco, for example, offers it to patients with early stages of AGA who refuse to take approved FDA therapy or complain of its side effects, or simply to those who would like to add a secondary therapy for AGA, even after being informed that PRP injections do not always achieve a positive effect. Drs. Cole and Rinaldi offer it to anyone provided they are good candidates (see ideal candidates above). Dr. Perez-Meza offers it only to patients who do not respond to medical therapy or who do not wish to try any medical treatment including low level laser therapy. Dr. Niedbalski offers it as an alternative to medical therapy to patients who are too young for surgery and who are non-compliant/intolerant of medication. Dr. Bob Reese performs PRP injections only during hair transplantation, but not as a medical therapy for patients with AGA.

Editor Emeritus from page 5

Discussion

The few studies that have been conducted on PRP and hair loss have shown that it does appear to have positive effects on hair growth. PRP induces dermal papilla cell proliferation *in vitro*, induces angiogenesis via VEGF, and up-regulates Wnt-signaling proteins and beta catenin, all of which appear to have important roles in hair follicle activation.

The overall positive experience of serious “PRP experts,” including those whose opinion has been sought for this article, tempts us to consider trying PRP in our practices. However, caution is a must. The intervention has to be performed correctly, following the indications of those more experienced than us, and it is important to realize that until randomized, placebo-controlled, clinical studies have proven its efficacy (using objective tools for measuring hair growth), in the eyes of the scientific community PRP will continue to be regarded as a controversial form of therapy for hair loss.

The following are unsolved areas that, in my opinion, need to be addressed:

1. We need to standardize a protocol for PRP preparation. The number of different PRP devices on the market makes it difficult to compare the results.
2. Clinical research studies are needed to assess the concentration of platelets that are being injected into the tissue as well as the concentration of growth factors, correlating both with the clinical response.
3. Although experience and anecdotal clinical data are important, we still need randomized, placebo-controlled, clinical trials to be certain that PRP does in fact induce hair growth.

Let's keep PRP inside the scientific boundaries. Throughout its history, our field has been plagued by the invasion of “miracle” cures through hair potions and lotions. It would be sad to see PRP having a similar fate to these, becoming yet another trivial and short-lived form of untested “popular” therapy. ♦

Dear Members: The session at the 2013 Annual Scientific Meeting to which Dr. Jimenez refers was recorded and is available to members in the Members Only section of the ISHRS website at www.ishrs.org. See page 28 of this issue for details.



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Hair Follicle Cloning *from front page***New Approaches for Follicle Regeneration**

By expansion of dermal papilla cells by growth in culture, you are essentially taking them from a three-dimensional environment where they are surrounded by other cells, to a two-dimensional environment where they have plastic on one side and culture medium on the other. This results in a decrease in communication between the dermal cells, which likely contributes to their loss in specificity or identity in culture. Recently, we demonstrated that growth of cultured human dermal papilla cells in hanging drop cultures results in formation of three-dimensional dermal spheroids. We were able to show that dermal spheroids maintain their specificity after transplantation into human skin, where they are capable of inducing growth of de novo hair follicles, rather than contributing to the interfollicular dermis.¹¹ Moreover, 22% of genes expressed in intact papillae, whose expression was deregulated by normal culture growth, were restored by growth of dermal papilla cells in spheroids. This indicates that the microenvironment within dermal spheroids results in increased communication between cells, and a partial restoration of dermal papilla identity—enough to initiate the cascade of events leading to new follicle development. This being said, the molecular contribution of the epidermal cells to the interactive process has still to be elucidated.

Conclusions

Thirty years ago, we first demonstrated that cultured rodent dermal papilla cells could be used to induce new hair follicle growth.¹² We now know that hair follicle cloning is possible using human hair follicle cells. However, the hairs we have produced are quite small, directionally non-uniform, and it remains to be seen how long they will grow for and whether the follicles will cycle. Therefore, many reproducibility and engineering challenges still remain before conventional hair transplantation procedures will be replaced; however, we will continue to take lessons from biology, and by developing a better understanding of the properties of hair follicle cells we will, in time, be able to improve on this important proof of principle study.

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