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President's Message

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As we start a new year, many of us make resolutions about how we're going to improve our lives, our relationships, our businesses, and often even our weight. I expect 2013 to be a very productive year for the ISHRS and its membership. We are confronted with a few difficult challenges; however, I have great confidence that the ISHRS's history of cross specialty respect and cooperation, critical thinking, and open communication will overcome the challenges that confront us, and create a stronger profession.

As you know, the ISHRS is a certified provider of accredited CME through the Accreditation Council for Continuing Medical Education (ACCME). That means that our training programs have met the standards set by the ACCME to provide the highest quality physician education that satisfies the CME requirements of all U.S. state licensing boards as well as many other medical licensing bodies around the world. The new guidelines for building CME programs require that the program director identify specific gaps between "current practices" and "best practices" for specific tasks or issues that need to be improved, then train to encourage best practices behaviors. So, critical to retaining our ACCME accreditation are the definitions of "current practice" and "best practices" in hair restoration surgery. Past President Paul Cotterill has agreed to stay on as CME Committee Chairman and guide us through the ACCME recertification process that will take place over the course of this year.

For the ISHRS to meet these guidelines is no easy task, because ACCME is encouraging all of their accredited providers to set their best practices based upon evidence-based medicine. The ISHRS is a small society, whose members have few resources to fund these sophisticated medical investigations.

As Sherlock Holmes suggested, "It is a capital offense to theorize before one has data" (Sir Arthur Conan Doyle). Therefore, the ISHRS has embarked on a multi-year project to identify the gaps by using recognized experts in the field to define "best practices." To that end, Dr. Cotterill has created a subcommittee of recognized experts and educators in the field, tasked with defining "current and best practices" for hair restoration surgery through surveys of the membership.

The success of this program depends on as many of our members as possible participating in the surveys to make the data collected more reliable. We have a total of 14 surveys to complete and your participation is greatly appreciated.

The analysis of the first of these surveys—"Who Does What"—has recently been completed and summarized in a white paper soon to be published. This survey focused on another difficult challenge facing the specialty this year: the expansion of delegated tasks of technicians into donor harvesting. The CME Committee and ISHRS Board of Governors have published the position that donor harvesting is a "critical to quality task" that should always be performed by the physician. Trivializing the importance of carefully planned and skillfully executed donor harvesting, and balancing all the intra-operative variables associated with the task, is a serious disservice to the patient. Thinking that one can train a non-physician in donor area management and conservation in just a few days is nothing more than a demonstration of the naïveté of the instructor.

It is disappointing that some of our most respected hair transplant technicians have expanded their services into donor harvesting, a responsibility that is much more expansive than they realize, and an activity that in most jurisdictions is considered the practice of medicine without a license.

I would encourage all of our membership to not to allow this behavior, as your Board of Governors has been advised there may be serious legal risks not only to the hair transplant technician, but also the physician who facilitates the activity. Our goal as a professional society is to assist each member in providing the best possible patient outcomes.

Yes, we are confronted with significant challenges for 2013. But we're a strong organization composed of bright, well-meaning, patient-focused physicians. I'm sure we're up to the task. What's good for our patients is good for our membership and our society.◆

Co-editors' Messages

Nilofer P. Farjo, MBChB *Manchester, United Kingdom* editors@ISHRS.org

People often ask: "Why do you go to the annual ISHRS conference every year? Surely things don't change that much." And perhaps they don't, but it's the subtleties that make all the difference between a good transplant and a great one in my opinion. Someone asked me at the meeting which one has been my best meeting. I immediately replied: "This one." And that's not because it was in such a beautiful location. After all, who wouldn't want to go the Bahamas when you've had rain every day for 6 weeks in your own country? And it wasn't because the program was great. Paco Jimenez has to be congratulated on producing an excellent educational program. And in spite of the more relaxed atmosphere, the standard was as high as any previous meeting. There were a lot of FUE talks this year, but that goes back to my introductory line that it's the small things that make the difference. And it may be this ability to produce a transplant without a linear scar that for some patients is the convincing factor that makes the difference in deciding on having the surgery at all. That doesn't mean that we should wholeheartedly accept this technique as the new "old" standard (after all, punch grafting was going out of vogue when I trained). But we do need to do some critical evaluation and investigation to see how we can best move forward.

So I have digressed from my point of why this was the best meeting for me: The Platinum Follicle award. Receiving this award has to be the pinnacle of my career. It is an amazing feeling to get recognition from your peers. I imagine it's like being at the Oscar's where just being nominated is great recognition in itself. We did get a hint beforehand that one of our good friends and colleagues was going to nominate us, but we didn't think that we stood a chance. If you look at the names of the past winners and the body of research that they have produced, we didn't think that we would be chosen. All we do is have enquiring minds and dabble in a bit of research (because we have nothing better to do with our time—obviously very boring people). It was so amazing that, for a change, Bessam was speechless and I had to make up something on the spot. But as I have said in previous editorials: Once you stop asking questions such as "Why?" and "How can I improve?" then you might as well hang up your scalpel (or punch for that matter). Bessam and I don't consider ourselves to be terribly clever people, so we do need to continually educate ourselves. And maybe along the way we can help the scientists (the real brains) figure out what is going on in the hair follicle. Maybe we might even find a way of putting ourselves out of business. But I think that's still a long way off.

It is not just at ISHRS meetings that I learn something of significance to my daily work routine. I have just finished being a jury member in a trial. What did I learn from that? Well, it's very boring being a juror and nothing like it is on television. Most of your time is spent sitting around waiting for something to happen. I also learned that past experiences were important. Certainly in coming to my guilty verdict, I did have some insight into the implications of the medical evidence presented: my time in A&E wasn't wasted after all! I also learned that reading all those detective novels over the years was more or less a waste of time when it comes to detective work. Most of the detection happens by following boring routines and not by the glamour of sneaking around with flashlights in the dark. A bit like doing transplants, I suppose, although Bill Reed may disagree with me on this point. Following the tried-and-tested routines does produce results; but, unlike knitting (see his editorial), we shouldn't do it without looking at individual variability. So it is incumbent upon each of us—especially those involved in education—that we ensure our specialty is inclusive. When we see that there are doctors that need to improve their results because they haven't learned the basics, especially in terms of things like hairline design, then we should take on that responsibility of helping them get that education. No matter which donor removal method is used, without implementing the basic routine, the superb results that we know are possible will never be achieved.◆

William H. Reed, MD *La Jolla, California, USA* editors@ISHRS.org

With the effort entailed pulling together another issue of the Forum, comes an appreciation of the complexities of our specialty. I'm repeatedly struck by how far hair transplantation has come from when one outstanding surgeon in my hometown of San Diego did his surgery as just another procedure in his general dermatology practice and would extract and place 20 large plugs while having the patient apply pressure to control the bleeding. This is not to disparage this moment: in the 1970s it was as amazing and cutting edge as what we do today. Progress is made standing on the shoulders of such forefathers as this surgeon.

Fast forward, however, to the 21st Century to see where we chose to go from this vantage point. Our specialty has become specialized beyond just a procedure in a general dermatology practice and, as such, has become in most cases its own free-standing subspecialty that necessitates business and entrepreneurial acumen as well as surgical and medical skills. Our surgery has congealed around smaller and smaller incisions with many surgeons having expertise only in surgical wounds of less than 1mm width and 5mm in depth. That this is the magnitude of the surgery has resulted in people without medical degrees and even robots performing the procedure, and doing so with good results in some cases. The "surgical" has become ever less important than the artistry, judgment, and experience of the practitioner. If our specialty becomes too restricted in the manner in which it approaches hair loss, the resultant "surgical" requirement can more resemble needlepoint or knitting than surgery. This is not to cast dispersions upon the skill entailed in extracting a follicle with a punch; it still humbles me when I see the speed and dexterity that others have in comparison to me, but I can see it a stretch to consider it surgery that requires years of training in order to avoid trouble from the surgical perspective.

The medical aspects of our specialty can also be extraordinarily exciting and broad…or it can be as simple as prescribing minoxidil, since some could say finasteride has become too controversial, and no storage solution is necessary other than normal saline since it seems to have worked adequately in the past. Again, we can be a specialty that demands a physician's education, or it can be done by a robot.

This issue, occurring as it does with memories of our meeting in the Bahamas fresh in our minds, echoes the breadth and depth of

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INTERNATIONAL SOCIETY OF HAIR RESTORATION SURGERY

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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1. 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.
2. If results are presented, the medical regimen or surgical techniques that were used to obtain the results should be disclosed in detail.
3. Articles submitted with the sole purpose of promotion or marketing will not be accepted.
4. Authors should acknowledge all funding sources that supported their work as well as any relevant corporate affiliation.
5. Trademarked names should not be used to refer to devices or techniques, when possible.
6. 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.
7. Once the manuscript is accepted, it will be published as soon as possible, depending on space availability.
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9. 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.
10. 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).
11. We CANNOT accept photos taken on cell phones.
12. Please include a contact email address to be published with your article.

Submission deadlines:
February 5 for March/April 2013 issue
April 5 for May/June 2013 issue

Reed Message

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what our specialty can be. (Actually it would have been even more broad and deep if room had allowed.) From the lead article by Dr. Aby Mathew on molecular biology of holding solutions and Dr. Jeff Epstein's surgical artistry that continues the tradition of Drs. Shelly Kabaker and Mario Marzola to… well, I was going to list all of the articles that make the point of the medical and surgical breadth and depth but it would be far too long a sentence. Looking at the table of contents shows my point and to this point of the delightful complexity of our specialty should be added that they are contributions from you, my colleagues, acting collectively, sharing ideas and experience and the ISHRS has been the vehicle.

The combination of the necessary medical, surgical, and artistic skills makes ours an enviable profession. Let's keep refining our breadth as well as our depth. Let's welcome considerations of new ideas but subject them to continuing high levels of collegial, constructive criticism. Let's appreciate the skills required of the hairline advancement and tissue expansion techniques and strive to find the pros and cons of both strip excisions as well as FUE. And while we are at it, why not consider the ironies of our inconsistencies historically that put the multi-bladed knife and the multi-unit graft into the dumpster: many of us seem comfortable with transection rates for FUE that are no better than those that doomed the multi-bladed knife. Others are comfortable drifting out of traditional safe donor zones with the associated risk of revealing FUE donor scars, yet we're critical of the "unnaturalness" of multi-unit grafts even though they would never become visible except under close, deliberate scrutiny.

To survive we need to have some success as a businessman and entrepreneur, but let's make time in our professional life to keep alive the excitement derived from being both a physician and surgeon. By doing so, we will create excitement and satisfaction for ourselves and continue to refine the nuances of the medical and surgical aspects of our specialty. Enjoy reading the efforts of our colleagues contained herein.◆

Notes from the Editor Emeritus

Bernard Nusbaum, MD *Miami, Florida, USA* drnusbaum@yahoo.com

Medical therapy for hair loss: what we know and what we need to find out

I would like to review the status of current knowledge regarding what therapeutic options are available for treating hair loss patients with medical therapy. This topic is of importance to not only our physician readers who choose to treat some of their patients strictly with non-surgical modalities but also to all hair restoration surgeons as the ever present limiting factor for our procedures is the donor supply. This makes medical therapy a necessary adjunct to the overall treatment plan.

Current Knowledge

We know that minoxidil acts through the mechanism of opening potassium channels to increase the size of hair follicles as well as the percentage of anagen follicles.[1] The 5% concentration is superior to the 2% in treating male pattern hair loss (MPHL)[2] and although the package labeling states that it is indicated for treating the vertex, we have seen positive results in the top scalp and frontal areas. Topical minoxidil is extremely safe, as evidenced by its over-the-counter status in the United States, and side effects are predominantly allergic or irritant contact dermatitis, the latter being less frequent with the foam vehicle, which does not contain propylene glycol. In treating female pattern hair loss (FPHL), the 2% solution, which is marketed for women, is equally effective when used BID as compared to a single daily application of the 5% foam.[3] 5% minoxidil, however, is associated with a higher incidence of facial hypertrichosis in women than the 2%, but this side effect is reversible within 1-3 months after treatment is discontinued.

We have long observed that, in histologic sections, FPHL exhibits a greater degree of inflammatory infiltration than its male counterpart, and, additionally, recent studies have shown the presence of immunoglobulin deposition in FPHL using immunofluorescent staining.[4] My treatment results for women have improved since I routinely started to add a topical steroid to minoxidil in solution for enhanced efficacy in FPHL.

5-alpha reductase inhibitors, without question, are the most efficacious treatment for MPHL currently available. By inhibiting type II 5-alpha reductase, finasteride decreases both serum and scalp levels of DHT and increases hair diameter, growth rate, and, to a lesser degree, hair counts.[5] In large clinical trials of patients with MPHL, the most common side effects were decreased libido (1.9%), erectile dysfunction (1.4%), and decreased ejaculate volume (1.0%).[6]

In these large studies, as well as in many of our practices, we have observed that discontinuing the drug or lowering the frequency of administration results in resolution of the side effects. The rationale for lower or less frequent dosing stems from the fact that a 0.2mg daily dose (which represents 20% of the 1mg dose recommended daily) has shown 70-90% of the therapeutic effect seen with 1mg.[7] While I will not go into all of the reported post-marketing side effects, it is important to note that a small number of men claim that they have suffered permanent sexual dysfunction despite stopping the drug, and, in a small subset of men, prolonged sexual side effects lasting weeks to months have been reported.[8]

Questions Begging for Answers

While finasteride at a 1mg daily dose was shown to be ineffective in post-menopausal women,[9] several case reports have demonstrated efficacy in FPHL using 2.5-5.0mg daily.[10,11] While, theoretically, the slight increase in testosterone that results from the administration of the drug and its subsequent conversion via aromatase to estrogen should have no adverse effect, we currently have no large-scale studies evaluating safety data regarding the administration of finasteride to women. It is important to note, of course, that the drug is teratogenic and should not be administered to any woman who is planning to become pregnant. My concern, however, stems from the remarkably high incidence of breast cancer in the general female population and the possible legal risk that the appearance of a breast tumor in a woman taking finasteride poses. Certainly, it might be wise to at the very least take a family history regarding breast, ovarian, or uterine cancer to rule out potential high-risk patients.

With regards to low level laser/light therapy (LLLT), the ranks of the "believers" has been growing steadily. Unfortunately, we have only one study published in the literature where the use of a handheld device showed a statistically significant increase in hair counts.[12] While some of us have anecdotally observed the results obtained from the more recently developed helmet/hat devices, well-controlled clinical studies are needed to determine the relative efficacy of the these two forms of laser devices (handheld vs. hat). In addition, what can we tell patients who are already on minoxidil, finasteride, or both with regards to adding laser therapy to their regimen? How much further improvement can they expect? Considering that the increase in hair counts observed with LLLT is in the 15-20% range,[12] which is what is seen with minoxidil therapy, it is possible, yet still undetermined, whether these two modalities act via the same target so that an additive or synergistic effect would not be expected. Finally, what is the optimal frequency, power, and duration of treatment for LLLT in men and in women? Should MPHL be treated differently than FPHL?

Mesotherapy, consisting of scalp injections of pharmaceuticals and vitamins, has been presented at our meetings, and Rinaldi's study of 126 patients with FPHL injected with dutasteride, biotin, pyridoxine, and d-panthenol shows photographic improvement in 63% of patients as compared to 17.5% on saline placebo.[13] The concept of mesotherapy seems attractive as presumably delivering small amounts of active compounds directly into the follicle might avoid systemic side effects. In addition, the intermittent treatment schedule offers the prospect of improved compliance as compared to daily, messy topical applications, especially in women. Until

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Editor Emeritus

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we determine which compounds are truly active and at what concentrations their efficacy is optimal via this route, we must remain cautious regarding safety and efficacy of this treatment.

Finally, my patience is growing thin with patients asking me about clinics that offer "stem cell" therapy, when in fact, it is platelet rich plasma (PRP) that is being used. While the use of PRP for hair loss stems from reports of enhanced growth of transplanted follicular units,[14-16] there appears to be a rationale for using this treatment in hair loss patients as the multiple growth factors released upon platelet activation presumably have the potential to"turn on" hair follicle growth. While small studies do show accelerated wound healing in hair transplant surgery and one form of PRP is FDA approved for wound healing applications, the clinical data to support a direct effect on hair growth is limited. A recent study, which was marred by lack of tattooing to mark target sites, showed increased hair counts and hair shaft diameter with the use of PRP alone, and PRP with a controlled release carrier.[17] Given the numerous PRP preparations currently available, all differing in platelet concentration and mode of activation, further studies are needed to determine whether significant efficacy for hair loss can be achieved and what the optimum preparations and treatment frequencies are for this specific application.

While our limited armamentarium for the medical treatment of hair loss appears to be growing (we anxiously await the results of the bimatoprost study for scalp hair loss), there seems to be a lack of incentive for those who are marketing some of these new products to conduct large, well-controlled clinical trials. Ultimately, we as physicians are to blame as, due to competition and hype, we are quick to purchase and offer our patients the "latest and greatest" without demanding more rigorous evidence.

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Cellular biopreservation

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storage results in limited reduction of metabolic demands (cells/tissues) and higher rates of enzymatic and/or structural decay (cells/tissues, blood, plasma, proteins, nucleic acids). Refrigerated/hypothermic (2°-8°C) storage allows for greater reduction in degradation related to ischemia/hypoxia and enzymatic decay, based on the Q_{10} equation principle that most metabolic reactions will slow down approximately 50% for every 10°C decline in temperature from normothermic conditions.[3] However, the protective conditions of hypothermia may also introduce potential stresses. The same characteristics of hypothermia that confer protection for cells/tissues (reduced metabolic demand) result in inactivation of ATP-driven ion pumps that would normally maintain osmotic balance, membrane phase changes, organelle instability, free radical generation, water flux, and ultimately, the accumulation of stresses that may cause cell death via apoptosis, necrosis, and/or secondary necrosis.[8-21] These conditions are exacerbated by storage/holding media that typically consist of culture media or saline. Culture media and saline are osmotically characterized as isotonic/extracellular-like solutions; that is, their ionic balance is similar to normothermic extracellular fluid. While suited for maintenance of cells at normothermic temperature (37°C) in cell culture conditions that mimic native environments, these extracellular-like solutions do not balance the hyperosmotic cells during low temperature preservation, and hence amplify the preservation-induced stress for cells and tissues since both the cells and the extracellular-like holding solutions lack active and passive mechanisms for modulating the hyperosmotic state.

From the moment that a biospecimen (such as a hair transplant donor strip and grafts of follicular units) is obtained by removal from the native environment, a degradation process begins and stresses accumulate. *Ex vivo* hypothermic conditions are utilized as a first step for slowing the rate of degradation of grafts. Under normal conditions *in vivo,* cells are bathed in an extracellular medium that is high in sodium and low in potassium compared to the interior of the cell. Plasma membrane ion pumps, such as the Na^+-K^+ ATPase that maintains the sodium and potassium gradients, use much of the energy produced from oxidative phosphorylation. For example, the maintenance of the Na^+, K^+, H^+, and Ca^{+2} ion concentrations in nerve and kidney cells account for as much as 25% of the ATP utilized, and as much as 50% of available ATP is used in erythrocytes for ion balance.[22] As mentioned previously, the maintenance of the levels of key ions outside the cell counteracts the osmotic pressure resulting from intracellular proteins, macromolecules, and organelles.[23] Anaerobic hypothermia, which is inevitable during standard *ex vivo* strip/graft storage intervals, results in decreased ATP production and suppressed activity of the ion pumps,[24] even as hypothermic storage provides the benefits of decreased metabolic rate and reduced demand for ATP. As a result of hypothermia in the absence of an optimized preservation solution (such as when cells are in isotonic saline solutions), many ions diffuse along their gradients into and out of the cell. Consequently, there is often cellular swelling because of water movement into the cell that may ultimately result in cellular lysis.[25]

Glycolysis for energy production continues under hypothermic conditions, although at a highly reduced level. Glycolysis is blocked at several points due to the inactivation of regulatory enzymes such as glycogen phosphorylase and glyceraldehyde-3-phosphate dehydrogenase.[26] In addition, the enzymes of the citric acid cycle are known to undergo conformational changes as a result of hypothermia (pyruvate kinase, glutamate dehydrogenase, arginosuccinase). Due to low-level glycolytic activity and disruption of the citric acid cycle and oxidative phosphorylation, lactic acid production results, as well as generation of hydrogen ions. The resulting intracellular acidosis (and tissue acidosis) causes lysosomal instability and cellular degradation.[27]

Considering the multiple steps of a hair transplantation procedure, there are a number of points where the tissue and cells might be negatively affected due to stress factors. Prior to excision, epinephrine at the donor site causes anoxia. The donor strip, or follicular units, is physically extracted from native tissue. This interferes with nutrient/waste exchange from the normal blood supply. Tissue and cells that are normally protected from the outer environment are now exposed to atmospheric O_2/CO_2, different temperatures, and potential airborne pathogens. The cells and tissue would begin a degradation process, but this may be somewhat slowed temporarily by placing the tissues into a hypothermic condition. According to surveys conducted by Cotterill/ISHRS, this usually is achieved by placing the strip and grafts into chilled normal saline.[28] Some hair transplant physicians may utilize modified isotonic solutions such as Plasma-Lyte®, Lactated Ringer's®, DMEM, or William's E (although none of these isotonic-based solutions are utilized for effective cell/tissue biopreservation in other arenas such as regenerative medicine or organ preservation, and culture media such as DMEM/William's E may not fit the Quality/Regulatory footprint for clinical applications, especially if additives are utilized at point of care which represents a potential contamination risk). Slivers one to two follicular units thick are removed from the strip via microscopic dissection, which is often a period of warming due to the heat emitted from the microscope lamp. At this stage, slivers are often placed once again in chilled solution until they are again dissected under a microscope into individual follicular unit grafts. This periodic exchange between chilled storage and warming intervals can be a point of variable stress, especially depending on the number of grafts. Eventually, rewarming on the gloved hand of the physician pending placement varies from less than a minute to probably 5 to 8 minutes or more in some practices.[29] Finally, healing of the graft and revascularization to support long term survival is another point of variability and stress; for example, transplanted hair follicles may require ~3 days for revascularization to replenish the new blood supply.[30,31] During this entire process, there is also the potential for dehydration, transection, in-process blunt trauma, and ischemia reperfusion injury following implantation of the grafts.[32,33] A review of these steps within the hair transplantation procedure offers approximately a dozen points of potential stress to the tissues and cells. Limmer reported the steady decline in graft viability in correlation with graft storage time,[34] which was also reported by Kim and Hwang.[35,36] Cooley demonstrated the extensive generation of potentially harmful free radicals following graft reimplantation.[32] Krugugler reported the onset of DNA fragmentation, and the benefits of antioxidants within the storage solution.[37,38] Parsley and Perez-Meza nicely reviewed a wide variety of considerations related to the topic of strip/graft storage.[33] Other cell models have shown that similar stresses result in a variety of molecular responses within the cell, even for short periods, sometimes in manners that are not visible with simple viability assessment methods.[39,40] Any "stress" tends to elicit a response within the cell, with subsequent cell damage being dependent on whether the cell is able to "manage" the

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stresses (similar to our own ability to manage the cumulative impact of personal and physical stresses). Cellular and molecular responses that can be triggered by the various potential stresses within a routine hair transplant procedure may involve biochemical perturbations,[41-43] generation of cold shock proteins,[44] change in cell volume,[45] which can also affect the cell membrane, protein uncoupling,[46] unfolded protein response,[47] and various mechanisms of cell death from suboptimal biopreservation[11,13,48] and temperature variations[49] that manifest at the genomic, proteomic, mitochondrial, membrane, and enzymatic levels of the cell,[11,13,15,50-53] Simply, the more stresses on the cells and tissues, the less likelihood of cellular recovery and more likelihood of cell damage or death. Mechanisms of "buffering" or reducing the accumulation of stresses during the procedure would benefit graft recovery. (Note: We have not even touched on the impact of patient variability as a potential enhancer of cell stress yet; however, this is discussed below).

Focus on the Preservation Solution for Graft Holding

An effective cold storage solution utilized during the graft holding stage of hair transplantation requires components that will provide an optimal concentration of ions and impermeant molecules to maintain ionic and osmotic balance during hypothermic dysregulation of homeostasis.[8] An intracellular-like solution, as opposed to an isotonic/extracellular-like solution such as culture media or saline, is one that closely balances the altered cellular ion concentrations that result from and at hypothermic temperatures and nutrient deprived conditions that exist when cells, tissues, or organs are without normal blood supply at normothermic conditions. Aside from the ionic concentrations in an optimized preservation solution designed to balance the ions of cells during hypothermia, there are several additional considerations that are important to hypothermic preservation. The solution should contain impermeant molecules replacing chloride ion (Cl^-) in the extracellular space. The decreased activity of the Na^+ pump during hypothermia results in increased Na^+ levels within the cell, and subsequently results in increased Cl^- levels within the cell to maintain the balance of charges. The increased osmotic pressure within the cell causes an influx of water from the extracellular fluid that induces cell swelling and lysis (in the absence of an appropriate preservation solution). Glucose may serve as a source of energy during cold storage. However, excess glucose during this metabolically depressed and oxygen deprived state will favor the production of lactic acid, resulting in cellular acidosis.[54] Therefore, glucose should be present at very low levels. An effective buffer is also critical to the system. Normally, cells and tissues are buffered primarily by the bicarbonate buffer system. However, bicarbonate is not the most effective of buffers at low temperatures.[42,43] Finally, upon recovery from cold storage, energy substrates can aid the synthesis of ATP for the increased metabolic demands of the cell.[55] All of these considerations are important components of an optimized hypothermic preservation solution that is designed to maintain ionic and osmotic balance, prevent acidosis, and prevent cell swelling. With the evolution of optimized preservation media, the use of these solutions greatly advanced the field of organ transplantation, and this success has prompted the development and use of optimized preservation solutions for collection, storage, and transport of other biologic materials such as tissues and cells.

Traditional approaches to low temperature biopreservation often focused on the biophysical parameters of the system—ice management in frozen storage, cell volume, water content, etc. This approach laid a strong foundation for the science of preservation, and allowed for utilization of low temperature preservation as a tool for increasing the stability of cells and tissues. Further development of the science of biopreservation brought together the disciplines of cryobiology, cell biology, and molecular biology, as well as recognized that biopreservation methods involve many common stress pathways that result in biologically related mechanisms of cell death (apoptosis, necrosis, and secondary necrosis). This engineered approach for improved biopreservation (i.e., holding/storage) solutions for cells and tissues led to the development of the intracellular-like HypoThermosol® platform of low temperature preservation solutions that incorporates the discussion points mentioned previously such as balanced ions and impermeants, targeted pH buffering, potent free radical scavengers, and mitigation of apoptosis and necrosis.[8-21,56]

This improved biopreservation protocol of using an intracellular-like solution has been qualified by a number of regenerative medicine therapies including cardiac applications,[57-58] stem cell applications,[59] and dermal fibroblast applications.[60] To personify the difference to the cell between isotonic solutions (normal saline, Plasma-Lyte, Lactated Ringer's) and an optimized intracellular-like solution (such as HypoThermosol®-FRS), imagine being in Minnesota in the cold of winter; isotonic solutions (balanced for cells and tissues at 37°C/98.6°F) are like a tank top (saline) or T-shirt (Plasma-Lyte, Lactated Ringer's). A brief exposure to the cold may be uncomfortable, cause a cellular response such as the equivalent of "shivering," and may cause non-lethal damage. But wearing an insulated winter coat (optimized intracellular-like solution) makes the short exposure easier to bear, and is of more noticeable benefit with longer exposure to that cold exposure—or of more benefit to those (cells) more sensitive to the cold.

Figures 1 and 2 highlight the variability in preservation efficacy between an intracellular-like biopreservation solution and traditional isotonic storage media.

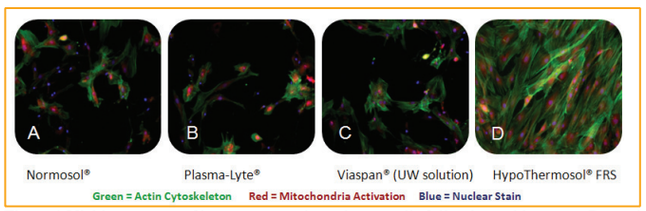

Figure 1. Viable recovery of human mesenchymal stem cells following cold storage. Dermal papilla cells share lineage with mesenchymal stem cells. Cell types relevant to hair transplant procedures, such as dermal fibroblasts and epithelial keratinocytes, also exhibit similar post-preservation results to the fluorescent micrographs above (fluorescent micrographs and data reference provided courtesy of BioLife Solutions, Inc.). Cells stored in Normosol (A), Plasma-Lyte (B), and Viaspan (C) exhibit lower cell number and less cell integrity following storage, in comparison to cells preserved in HypoThermosol-FRS (D).

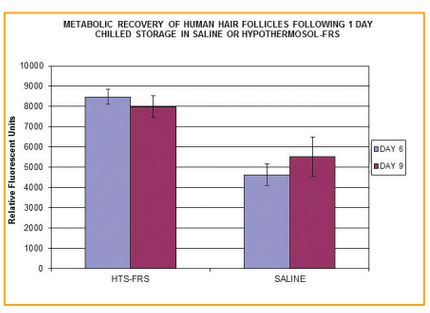

Figure 2. Differential recovery of metabolic activity in human hair follicles held in HypoThermosol-FRS or normal saline at 2°-8°C. Quantitative readings of a fluorescent metabolic indicator from follicles assayed after Day 6 and Day 9 recovery in cell culture conditions following 1 day of cold storage. Note the lower metabolic viability of follicles stored in saline even a week following the cold storage hold interval.

Graft Holding Temperature

A number of discussions within the hair transplant arena have also focused on the appropriate strip/graft holding temperature. Some have reported adequate graft recovery following ambient storage,[33,35,36] some have advocated using a temperature that straddles the fine line between freezing temperatures and non-freezing temperatures (0°C),[62] and many methods utilize the chilling temperature of 4°C.[32,33,63,64] It is of noted interest that positive graft survival results have resulted from a wide range of temperature (0°C to ambient), however, there is certainly variability in the interpretation of graft "quality" within the hair transplant arena. As mentioned above, hypothermic temperatures offer the advantage of reducing metabolic degradation and reducing stress related to hypoxia/ischemia, similarly to that demonstrated in the organ preservation field. Beehner also reported a reduction in blunt trauma of the follicles associated with hypothermic temperatures.[65] This is logical as the use of hypothermia likely limited the cumulative stresses occurring in grafts. Due to the difficulty in realistically maintaining an exact temperature under various conditions of hold or transport, industry cGMP (US FDA Current Good Manufacturing Practices) cold management tends to utilize the range of 2°-8°C with a target point set as 4°C when referring to hypothermic preservation of cells and tissues. The concept of utilizing 0°C is certainly within logical reason if one appreciates the use of hypothermia to suppress metabolism. There are two considerations when dealing with 0°C as a target for cold storage. First, the reason that 4°C is considered "optimal" for hypothermic preservation of cells is because water has its maximum density of 1g/cm^3 at 4°C, which then may affect osmotic fluctuations. Secondly, 0°C has the potential for ice crystal formation if temperature fluctuates below 0°C. Metabolically, 0°C will slightly lower the metabolism compared to 4°C but not by much (comparative range of 5% vs. 3.75% of normothermic metabolism), and it could be argued that the increased risk of slight temperature fluctuations below freezing that could cause damaging ice crystals may not be worth the risk.

ATP within Hair Transplantation

Recently, there has been growing interest in and reports of the utilization of ATP (adenosine triphosphate) as part of hair transplant procedures. This is an avenue of logical targeted intervention, as some of the damaging effects of ischemia/hypoxia are based on the inability to support maintenance of cellular energy. However, it should be noted that not all forms of delivering ATP to the cell are equivalent in efficacy. The ATP molecule itself is only capable of relatively short stability, especially during preservation. Therefore, effectively delivering ATP to the cell is also due consideration. Cooley[66] and Beehner[67] have reported promising results from long-term cold storage of grafts that were maintained in the combination of an intracellular-like preservation solution (HypoThermosol-FRS) and a liposomal embodiment of ATP. The novel liposomal ATP is based on Ehringer's work at the University of Louisville[68] where the ATP is packaged within liposomes (lipid membranes) that are able to fuse with the cell's own bilipid membrane to efficiently deliver ATP into the cell. Further studies are warranted, however, this promising combination method brings together one method of reducing stress during graft storage (intracellular-like HypoThermosol media) with another method that targets a cellular deficiency during and after graft storage (effective delivery of ATP inside the cell). Cooley showed that in an enhanced stress model to highlight potential avenues for cellular preservation, grafts stored in saline resulted in 0% survival, and grafts stored in HypoThermosol-FRS demonstrated 44% survival.[66] This comparison alone highlights the benefits of improved biopreservation using an intracellular-like holding solution. Furthermore within this same single-patient study, Cooley reported that grafts stored in the combination of HypoThermosol-FRS and liposomal ATP exhibited 72% survival. Figure 3 highlights the visual results reported by Cooley in this single-patient study for the HypoThermosol®FRS + liposomal ATP sample set.

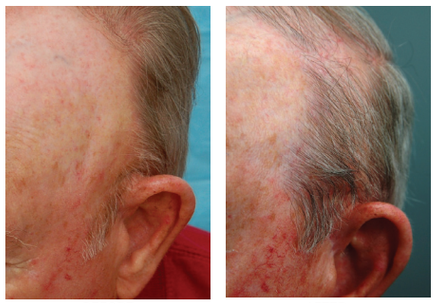

Figure 3. Efficacy of hair follicle transplant following 5 days of storage at 4°C in HypoThermosol + liposomal ATP. A single patient study was undertaken in a 71-year-old man, who had both male pattern hair loss and a large area of alopecia on the left temple as a result of radiation for skin cancer. Donor hair was harvested from the occipital scalp, dissected, and stored for 5 days at 4°C. Afterwards, grafts were implanted into alopecic areas (1,200 grafts). The patient was seen at 3, 6, and 9 months for evaluation and final hair counts were performed at 12 months.

Further Summary Considerations

Hair transplant physicians operate under the practice of medicine with appreciable flexibility within their clinical methods. Hair transplant physicians can also appreciate an aspect of what they do as a form of regenerative treatment for the patient (with each ISHRS Annual Meeting there also seems to be increased profile of the cell-based hair regenerative medicine treatments from groups such as Intercytex, Aderans, RepliCel, etc.). Within the regenerative medicine field (cell therapy and tissue engineering), regulatory agencies provide guidance for the use of current Good Manufacturing Practices (cGMP). One aspect that is advised within cGMP is risk management, where assessment and mitigation of potential system risks is mandated to ensure appropriate quality of the manufactured end therapeutic product. Similarly, within a hair transplant procedure, one inherent goal is to maximize the quality of the transplant (graft survival, hairline design, graft density, scalp irritation/edema, rate of regrowth, hair shaft thickness, etc.). As mentioned above, there are multiple points of stress within a procedure that could affect the transplant quality and there are a variety of holding/storage solutions utilized for in-process graft holding. This one step in the transplantation procedure is worth further examination to assess whether clinical practice and current methods are aligned with cGMP guidance for risk mitigation and management. Again, the improved biopreservation protocol of using an intracellular-like solution has been qualified by a number of regenerative medicine therapies including cardiac applications,[57-58] stem cell applications,[59] and dermal fibroblast applications.[60]

As noted by Parsley and Perez-Meza, there may be some discrepancy between the often-reported high graft survival rates and the overall actual percentage of graft survival across the variable patient pool.[33] In the best examples used of patients with >90% graft survival, there may be limited room for perceived or actual improvement. However, there are several considerations to weigh. If the patients who do not enter the procedure with the

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best quality scalp, donor tissue, and health have the potential to bring pre-existing stresses into the procedure even before the first extraction, there is the strong possibility that the further stresses outlined above during the procedure may result in cumulative stresses that compromise the quality of the transplant. Even with rigorous patient screening, it may be difficult to fully know the variable preconditions (intangibles) a patient brings into the procedure that might negatively impact the quality of the transplant. Therefore, any potential stress mitigation steps (such as improved holding solutions) may reduce the likelihood of an unsatisfied patient. The strip/graft holding steps of the hair transplantation procedure are of critical importance to the success of the hair transplant. This review highlights much information that has been accumulated regarding cell, tissue, and organ biopreservation, with many topics still for further scientific examination. The lessons from organ transplant and regenerative medicine offer insight toward methods of using optimized intracellular-like hypothermic storage media for improved graft *ex vivo* preservation as one step in continuously maximizing patient outcomes.

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