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A review of cellular biopreservation considerations during hair transplantation

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Introduction

Appropriate clinical biopreservation of cells and tissues is a critical factor in hair transplantation procedures, as well as in regenerative medicine (cell therapies and tissue engineering) and organ preservation. During the course of a hair transplantation procedure, the cells and/or tissues experience multiple forms of stresses related to the procedure from before the donor strip and/or follicular units are extracted, through the dissection and graft holding stages, and past the point of re-implantation. There are certain cellular and biochemical aspects of the hair transplant model that are unique to the specific types of cells and tissues involved. However, there is also much that can be taken into consideration from other *in vitro* and clinical cell models relevant to regenerative medicine, as well as from existing organ/tissue preservation knowledge.

Under normal conditions, the environment of human cells and tissues consists of an isotonic osmotic balance of ions that is maintained by ATP-driven cell membrane pumps. Major ionic constituents include sodium, potassium, calcium, and magnesium—each regulated by membrane pumps/channels that, along with chloride, are actively pumped inside or outside the cell to counterbalance the osmotic pressure of non-permeable fixed molecules inside the cell and subsequently regulate the passive flow of water into and out of the cells. In this manner, although highly simplified, there is an intracellular milieu and an extracellular milieu that are distinctly different from one another. Under normothermic conditions (37°C, appropriate balance of oxygen/carbon dioxide, exchange of nutrients/wastes, etc.), the fluid bathing the cells and tissues is isotonic, or also referred to as extracellular-like. The normothermic flow of nutrients through the cellular metabolic pathways fuels the production of ATP (adenosine triphosphate) that, in turn, drives the membrane ion pumps to maintain the osmotic balance. Cellular waste products are expunged from the cells, and free radicals generated by normal metabolism are removed from potential negative impact by the cell's antioxidant mechanisms. Only once these basic cell processes for maintaining "life" are in working order can the cell's energies be directed to further functional cellular processes specific to that cell's "job."

When cells, tissues, and organs are disconnected from this "normal" set of conditions even for a short time, there are many potential unbalanced states leading to detrimental consequences. Absence of nutrients (glucose, oxygen, etc.) deprives the cells of the raw fuel components needed to generate the cell's refined fuel, ATP. Short-term interruptions to the cell energy cycle can be compensated with derivation of cellular energy via the lactic acid cycle but cannot be maintained indefinitely. However, it is important to appreciate that the overall cell machinery is a highly complex engine of parts and pathways that generates intermediate compounds from specialized reactions at specific steps, and that there are aspects of this cellular engineering that cannot currently be artificially replicated in the laboratory or by cell culture media. Furthermore, it is also important to remember the critical functions of waste removal and gas exchange, which often require specialized laboratory equipment to effectively compensate in the absence of the natural cellular mechanisms. Therefore, current attempts to replicate out-of-body normothermic conditions have the potential to be incomplete and suboptimal.

Cellular and Molecular Considerations for Biopreservation

Biopreservation can be described as processes that suppress degradation of biologics for the post-preservation recovery of structure, viability, and function.[1,2] Hypothermic storage (primarily 2°-8°C) has been the preferred practical mechanism for storing cells, tissues, and organs for short periods of time (such as applicable in typical hair transplantation procedures). The ability of hypothermia to suppress metabolism is the key to maintaining cells, tissues, and organs under ischemic conditions.[3] The beneficial properties of hypothermia have been appreciated for a number of years. For example, in 1939, surface cooling of ischemic limbs was found to confer preservation ability for rat limb survival.[4] In the 1950s, hypothermia played an important role in the development of cardiopulmonary bypass surgery,[5,6] and in 1969 the demonstration that cold storage was an effective means of kidney preservation[7] stimulated the development of cold storage solutions for the purpose of organ preservation. Ambient

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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.

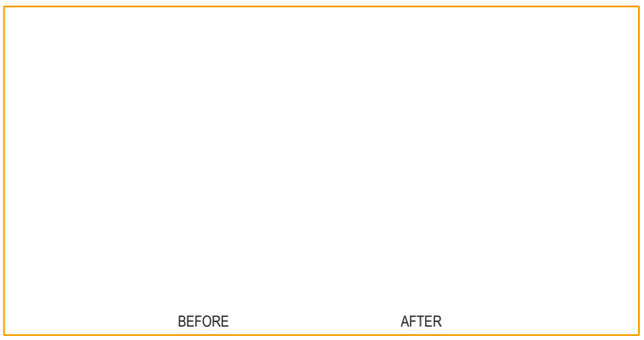

Figure 3. Efficacy of hair follicle transplant following 5 days of storage at 4°C in HypoThermosol + liposomal ATP. A single patient study was undertaken in a 71-year-old man, who had both male pattern hair loss and a large area of alopecia on the left temple as a result of radiation for skin cancer. 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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