Impact of Preservation Solutions on the Trichogenicity of Hair Micrografts Ascertained by Dermal Papilla Gene Expression

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BACKGROUND Appropriate storage of human hair follicle (HF) grafts during follicular unit excision (FUE) is crucial toward successful hair shaft implantation. Several commercial storage solutions are currently used to ensure ex vivo maintenance of follicular grafts viability and trichogenicity. However, quantitative experimental evidence demonstrating molecular changes in HF cells associated with the usage of different storage solutions is largely missing.

OBJECTIVE To identify gene expression changes in HF cells caused by ex vivo storage of hair grafts in different preservation conditions.

METHODS The authors performed gene expression analysis in dermal papilla (DP) isolated from HF stored under different temperatures and solutions. The expression signature of key genes controlling hair growth and cycling, apoptosis, inflammation, and senescence was assessed for (1) chilled versus room temperature (RT) and (2) DP cell medium, saline, Hypothermosol, platelet-rich plasma, and ATPv-supplemented saline.

RESULTS The authors found chilled versus RT to prevent inflammatory cytokine signaling. Under chilled conditions, ATPv-supplemented saline was the best condition to preserve the expression of the trichogenic genes *HEY1* and *LEF1.*

CONCLUSION Data disclose DP gene expression analysis as a useful methodology to ascertain the efficacy of preserving solutions and elucidate about the best currently available option for FUE clinical practice.

The authors have indicated no significant interest with commercial supporters.

Follicular unit excision (FUE) is a minimally invasive approach to transplant hair, highly popular and effective to treat androgenic alopecia (AGA), the predominant type of hair loss. Nonetheless, FUE still faces some challenges, such as the limited donor hair availability, rate of hair transection, and grafts' fragility.[1] Moreover, this technique requires a meticulous microsurgical work, during which the follicular units (FUs) undergo prolonged storage (5–10 hours) before retransplantation.[2] Previous studies reported the impact of time and storage conditions on grafts' viability.[2,3] The effect of different chilled isotonic solutions has been evaluated based on qualitative morphological evaluation of the grafts.[4,5] More recently, gene expression of key dermal papilla (DP) determinants after William E medium (for research use only; not approved for clinical use) storage at 37°C has been ascertained.[6] Albeit providing extracellular ionic balance, isotonic solutions poorly mimic the follicle microenvironment, lacking nutrients and oxygen. To circumvent this, some solutions became increasingly popular, such as HypoThermosol (BioLife Solutions, Bothell, WA) and liposomal ATP (ATPv; Energy

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.dermatologicsurgery.org).

ISSN: 1076-0512 • Dermatol Surg 2019;45:1649–1659 • DOI: 10.1097/DSS.0000000000001895

Delivery Solutions, Jeffersonville, IN) among others. HypoThermosol is an intracellular-like preservation solution claimed to protect cells from chilling stress. ATPv has been suggested as an energy supplier in blood-deprived environments. Recently, autologous platelet-rich plasma (PRP) has been used as adjuvant therapy during AGA restoration surgery, as it contains bioactive factors that promote neovascularization,[7] DP cells proliferation, and hair follicle (HF) growth.[8] However, quantitative data demonstrating the potential benefit of PRP and bio-enhanced solutions during hair graft storage is still missing.[9]

Hair follicle cycling consists of phases of growth (anagen), apoptotic-driven regression (catagen), rest (telogen), and shedding (exogen), which are tightly regulated by several signaling cascades.[10] At anagen, *Wnt* activation results in the stabilization of β-catenin, followed by triggering of the Tcf/Lef1 family of transcription factors.[11] Wnt/β-catenin signaling in the DP regulates *FGF*, *IGF*, *TGF*β, and *PDGF*, which mediate the instructive signals into hair follicle stem cells (HFSCs).[12,13] *Notch* is another essential signal transduction pathway to instruct HFSC,[14] cross-talking with *Wnt* during stem cell fate determination.[15] Specifically, *HEY1* is a downstream target of *Notch* signaling essential for DP trichogenicity.[16] Furthermore, ALP activity and *VCAN* expression are DP intracellular signaling modulators, particularly upregulated in anagen.[17,18] Conversely, anagen phase is characterized by downregulation of *Wnt*-counteracting BMP signaling molecules (*BMP2/4*). Anagen-to-catagen transition can be triggered by an increase of *JAK/STAT* pro-inflammatory signaling, which turns off *IGF1/IGF1R* signaling.[19,20] Inflammatory cytokines have also been described to induce catagen.[21] It is the balance between activator/inhibitor pathways that regulates cyclic HF regeneration by switching DP and HFSC between an active anagen phase and a degenerative catagen phase.

Here, the authors carried out a gene expression analysis of key regulators of HF growth and cycling to measure the impact of different storage solutions, used during hair transplantation, in the intact/fully inductive DP molecular signature.[20]

Materials and Methods

Micrograft Biopsies

The study enrolled 38 Caucasian male patients aged between 18 and 55 years, who gave their informed consent. Twenty to 30 micrografts were harvested from the occipital scalp of patients undergoing FUE. Hair follicles were obtained using a 0.95-mm micropunch (Scharf CE, Istanbul, Turkey). Anagenic HFs were selected under a stereomicroscope accordingly to stage classification as described in Ref. 22.

Hair Follicle Storage

Six FUs from each patient were immediately dissected after surgery, and HF bulbs were subjected to total messenger RNA (mRNA) extraction for quantitative polymerase chain reaction (qPCR) analyses (T0h—control group). The remaining FUs ($n \geq 14$) from each patient defined Experimental Groups 1 and 2, aiming to test the effect of different storage temperature and solutions on micrografts viability, respectively. To address the effect of temperature, $n \geq 6$ FUs were stored under the following conditions: saline solution (AGA, Lisbon, Portugal) and Human Hair Follicle Dermal Papilla Cell Growth Medium (DPC medium; Cell Application, Inc., San Diego, CA) at 4°C and room temperature (RT) (Experimental Group 1). To address the effect of different storage solutions, $n \geq 6$ FUs were immediately stored in saline solution, DPC medium, HypoThermosolFRS (Biolife Solutions), HypoThermosol supplemented with liposomal ATP solution (ATPv) (Energy Delivery Solutions) (1:10 dilution), ATPv-supplemented saline, or autologous PRP and kept in a fridge at 4°C for 6 hours (Experimental Group 2) (Table 1). Stored grafts were then subjected to mRNA extraction for qPCR.

Preparation of Activated Platelet-Rich Plasma

Autologous human serum was obtained from 7 patients undergoing FUE. Ten milliliters of PRP was obtained from 50 mL of whole blood using Skin.pras 50 kit (Proteal; Bioregenerative Solutions, Barcelona, Spain).

Messenger RNA Isolation

Messenger RNA was isolated from fresh HF grafts using the Qiagen RNeasy Kit (Qiagen, Hilden,

TABLE 1. Storage Solutions Tested in This Study

		Temperature			
Solution	*Description*	*RT*	*4°C*	*Observations*	*Figure*
Saline solution	Isotonic extracellular balance solution	+	+	*HEY1* repression; *STAT2* and *FOXP1* upregulation	Figures 1 and 2
DP cell medium	Isotonic extracellular balance solution + growth factors	+	+	Inflammatory gene upregulation (*STAT2*, *IL6*)	Figure 2; (See **Supplemental Digital Content 1**, Figure 1, http://links.lww.com/DSS/A138)
Human PRP	Contain autologous nutrients and growth factors	−	+	Growth signaling (*FGF7*, *KI67*); *IL6* upregulation	Figure 4
HypoThermosol FRS (BioLife Solutions)	Optimized hypothermic preservation solution; intracellular-like balance	−	+	DP inductive repression (*HEY1*, *WNT10B*); inflammatory gene repression (*IL6*, *STAT2*)	Figure 3
ATPv-supplemented saline	Energy source supplement	−	+	Growth signaling (*IGF1R*, *FGF7*, *TGFβ*, *KI67*)	Figure 5
ATPv-supplemented HypoThermosol FRS		−	+	Growth signaling (*FGF7*, *TGFβ*); *IL6* upregulation	See **Supplemental Digital Content 4**, Figure 3, http://links.lww.com/DSS/A140

DP, dermal papilla; PRP, platelet-rich plasma; RT, room temperature.

Germany) and reverse transcribed with iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer's protocol.

Quantitative Real-Time Polymerase Chain Reaction

Quantitative polymerase chain reactions were performed using iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories). All primers were designed in order to span at least one exon–intron junction (See **Supplemental Digital Content 1**, Table, http://links.lww.com/DSS/A137). Transcript gene expression was quantified by $2^{-\Delta\Delta Ct}$ method, where $-\Delta\Delta Ct = \Delta Ct$ (T6h) $- \Delta Ct$ (T0h), and *GAPDH*, *ACTB*, and *UBQ* were used as reference genes.

Trypan Blue Viability Staining

Hair grafts were immersed in 0.1% trypan blue (Gibco; Thermo Fisher Scientific, Waltham, Massachusetts) for 1 minute. Images were collected under brightfield stereomicroscopy, with a Plan Apo 1× objective (Leica Microsystems, Wetzlar, Germany).

Statistical Analysis

Sample sizes and statistical tests used in each experiment are indicated in figure legends. Statistical analyses were performed with Prism GraphPad v6.00 (Prism, CA). Statistical significance was considered for $*p \leq .05$, $**p \leq .01$, $***p \leq .001$, and $****p \leq .0001$.

Results

Effect of Storage Temperature on Hair Micrografts Gene Expression

For each donor, 6–7 follicle bulbs were profiled for gene expression under 3 conditions: (1) no storage (T0h), and storage in saline solution for (2) 6 hours at 4°C (T6h 4°C) and (3) 6 hours at room temperature (T6h RT). Expression profiling was done for: DP markers (*ALP*, *HEY1*, *LEF1*, and *VCAN*), hair matrix growth factor receptor (*IGF1R*), hair germ quiescence marker (*BMP2*), apoptosis marker (*BIM*), and pro-inflammatory cytokine *IL-1β*. Overall, the authors found that 6 hours of storage in saline does not

significantly alter the gene expression signature (Figure 1), with the exception of *VCAN* and *IGF1R* at RT (0.63-fold increase and 0.24-fold decrease, respectively). Although apoptosis and inflammation markers at RT tend to increase, no significant change was observed. However, when using DPC medium and comparing the effect of RT versus 4°C, an increase in inflammation and apoptosis markers was found at RT, as well as significant repression of the trichogenic gene *HEY1* (See **Supplemental Digital Content 2**, Figure S1, http://links.lww.com/DSS/A138). This suggests that lower storage temperature acts to prevent inflammation in both saline and DPC storage solutions, being significantly advantageous in DPC medium, where it also preserves trichogenic gene expression.

Impact of Different Holding Solutions on Hair Grafts Gene Signature and Viability

Next, the authors asked whether distinct holding solutions could differently compromise bulb cells' viability and trichogenicity. At the preferred temperature of 4°C, gene expression analysis was performed comprising key DP markers (*ALP*, *HEY1*, *LEF1*, *VCAN*, and *WNT10B*), the dermal sheath marker *αSMA*, and DP and hair matrix mitogenic/growth factors (*IGF1R*, *FGF7*, *FGF10*, *TGFβ2*, *KI67*, and *PDGFA*). In addition, pro-inflammatory molecules (*IL1β*, *IL6*, *STAT2*, and *STAT3*), hair germ quiescence markers (*BMP4*, *BMP6*, and *FOXP1*), and apoptosis and senescence-related genes *BIM* and *CDKN1A/p21*, respectively, were also assessed. Alterations on these main signaling pathways were investigated after exposure of hair micrografts to common holding isotonic solutions (saline and DPC medium), as well as autologous PRP, Hypo-Thermosol, and liposomal ATP (ATPv).

First, hair grafts from the same patient ($n = 7$ patients) were equally distributed and stored in saline and DPC medium isotonic solutions at 4°C. Saline stored grafts exhibited 0.44-fold decrease ($p < .01$) in the expression of the trichogenic determinant *HEY1* (Figure 2A). Note that *HEY1* expression was not compromised in the sample of patients ($n = 4$) shown in Figure 1A, suggesting that a larger sample size improves the data robustness. In contrast, DPC medium did not negatively affect DP markers. Expression of *αSMA* dermal sheath marker and *KI67* proliferation marker was increased in saline (*αSMA*, 0.27-fold, $p < .01$; *KI67*, 0.48-fold, $p < .05$) and DPC medium (*αSMA*, 0.47-fold, $p < .01$; *KI67*, 0.44-fold, $p < .05$), indicating a proliferative status of hair matrix and outer root sheath (ORS) cells in both isotonic solutions (Figure 2A,B). The expression of pro-inflammatory *STAT2* was also increased in saline (0.30-fold, $p < .05$) and DPC medium (0.43-fold, $p < .05$) (Figure 2C). An increase in *FOXP1* during saline storage was also noticed (0.42-fold, $p < .05$); however, it was not accompanied by an increase in other quiescence markers (Figure 2D). Moreover, significant 0.67-fold increase was also found for the extracellular matrix protein

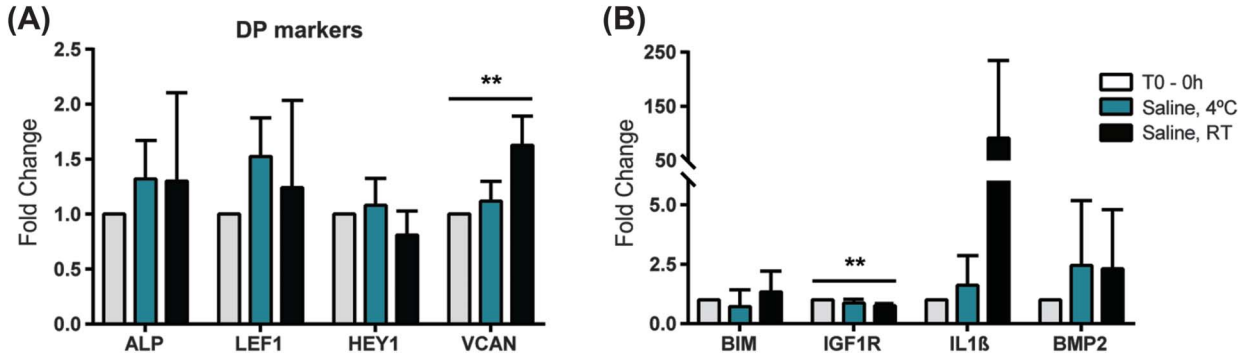

Figure 1. Impact of temperature storage on gene expression signature of hair follicle bulbs. Hair follicle bulbs were stored under different temperatures (4°C vs RT) for 6 hours. Three experimental conditions (no storage, storage in saline at 4°C, and storage in saline at RT) were tested for each patient ($n = 4$ patients). The expression of (A) DP marker genes (*ALP*, *LEF1*, *HEY1*, and *VCAN*), as well as of (B) hair germ quiescence (*BMP2*), hair matrix growth factor receptor (*IGF1R*), apoptosis (BIM), and inflammation (IL-1β) marker genes, was measured by qPCR. Gene expression fold change was compared to freshly isolated hair bulb controls of each patient (T0 − 0 hour). Data are presented as mean ± SD; $^{**}p < .01$ by Student unpaired *t*-test statistical analysis. DP, dermal papilla; qPCR, quantitative polymerase chain reaction; RT, room temperature.

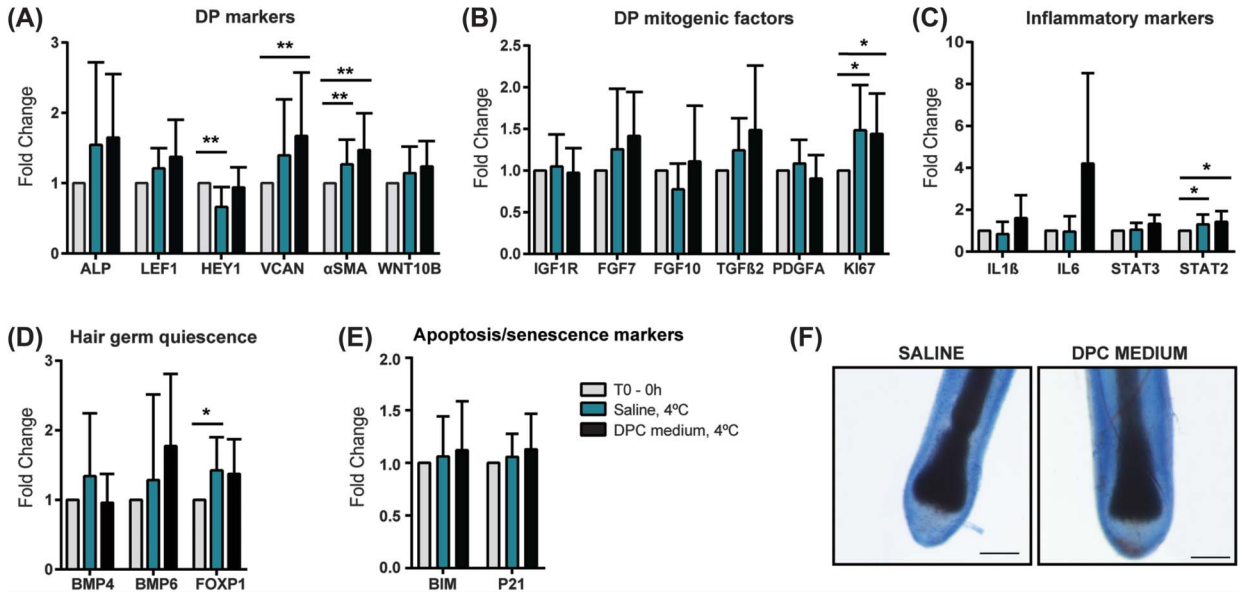

Figure 2. Impact of saline versus DPC medium storage on gene expression signature of hair follicle bulbs. Micrografts from 7 patients were stored in saline and DPC medium at 4°C. (A–E) Gene expression analysis of key DP marker genes, as well as of DP mitogenic signaling, pro-inflammation, hair germ quiescence, and apoptosis/senescence. Gene expression was measured by qPCR after 6 hours of storage, and fold change was compared with freshly isolated hair bulbs (T0 − 0 hours). Data are presented as mean ± SD. *$p < .05$; **$p < .01$, by Student unpaired *t*-test statistical analysis. (F) Hair grafts' viability after 6 hours of storage in DPC medium. Viability is inversely correlated with the blue staining. Saline stored grafts were used as control. Scale bars, 100 μm. DP, dermal papilla; qPCR, quantitative polymerase chain reaction.

versican (*VCAN*; $p < .01$) in DPC medium stored grafts. Finally, no significant changes were observed for apoptosis and senescence markers in both conditions (Figure 2E). To further ascertain the effect of saline versus DPC on micrografts viability, a trypan blue exclusion test was performed. No striking differences were observed ($n = 3$, Figure 2F), with both solutions compromising extensively the viability of the ORS cells.

Impact of HypoThermosol on Hair Grafts Gene Signature and Viability

To investigate for the potential benefit of HypoThermosol, gene expression analysis was carried out in 7 patients. Significant decrease in gene expression was observed for the DP determinants *HEY1* (0.31-fold, $p < .05$) and *WNT10B* (0.23-fold, $p < .01$), mitogenic factor *PDGFA* (0.24-fold, $p < .05$), pro-inflammatory molecules (*IL6*, 0.33-fold, $p < .05$; *STAT2*, 0.28-fold, $p < .05$), and *p21* (0.29-fold, $p < .01$). No significant changes were found for other genes involved in DP mitogenic signaling or hair germ quiescence (Figure 3A–E). Trypan blue viability assay revealed no apparent differences between saline and Hypothermosol storage ($n = 3$, Figure 3F). Altogether, the data show that Hypothermosol does not bring beneficial effect over saline or DPC medium, besides lowering the expression of *STAT2* inflammatory marker (0.72, $p < .05$), which was significantly upregulated in saline and DPC medium.

Impact of Autologous Platelet-Rich Plasma on Hair Grafts Gene Signature and Viability

To elucidate the putative benefit of autologous PRP, hair bulbs from 7 patients were profiled for gene expression. The authors found significant decrease in the expression of the anagen marker *VCAN* (0.20-fold, $p < .05$), but the expression of *FGF7* and *KI67* proliferation markers was significantly increased (*FGF7*, 0.35-fold, $p < .05$; *KI67*, 0.40-fold, $p < .05$). Also, a significant increase was observed for the pro-inflammatory cytokine *IL6* (1.21-fold, $p < .05$), whereas the hair germ quiescence marker *BMP4* was decreased (0.26-fold, $p < .05$) (Figure 4A–E). Trypan blue staining showed that grafts stored in PRP were markedly lighter blue in comparison to grafts stored in saline, thus suggesting that PRP ensures higher viability during storage at 4°C than any other solution aforementioned ($n = 3$, Figure 4F). In addition, to better mimic the clinical procedure, gene expression profiling

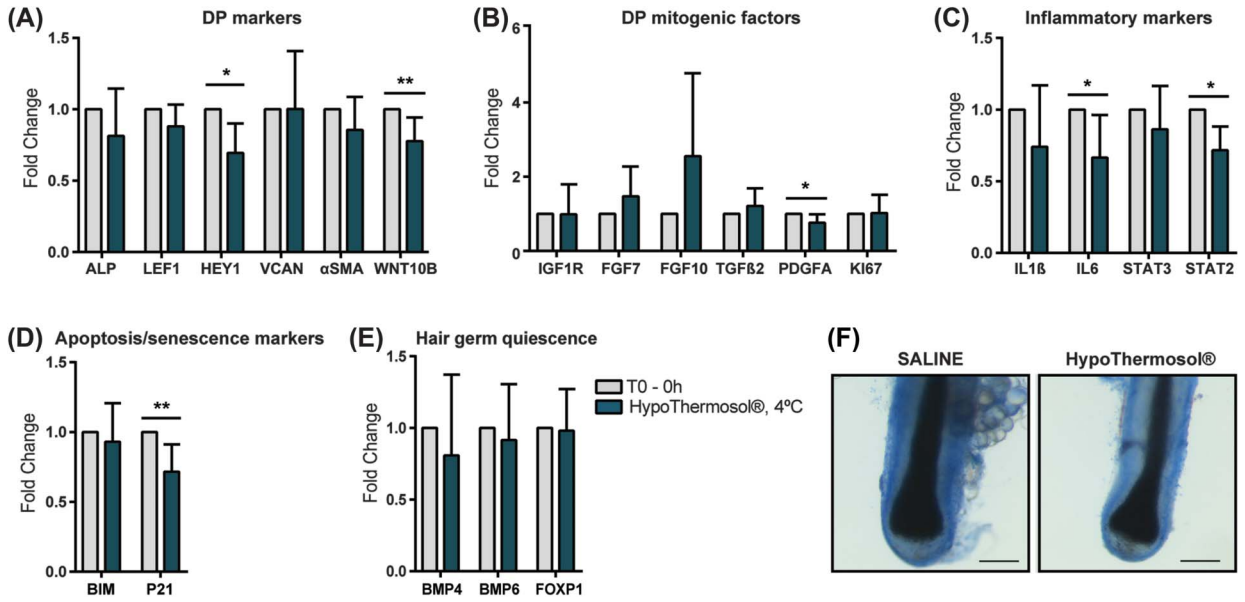

Figure 3. Impact of Hypothermosol storage on gene expression signature of hair follicle bulbs. Micrografts from 7 patients were stored in HypoThermosol at 4°C. (A–E) Gene expression analysis of DP marker genes, as well as of DP mitogenic signaling, pro-inflammation, apoptosis/senescence, and hair germ quiescence. Gene expression was measured by qPCR after 6 hours of storage, and fold change was compared to freshly isolated hair bulbs (T0 − 0 hours). Data are presented as mean ± SD. *$p < .05$, **$p < .01$, by Student unpaired *t*-test statistical analysis. (F) Hair grafts viability after 6 hours of storage in Hypothermosol solution. Viability inversely correlates with the blue staining. Saline stored grafts were used as control. Scale bars, 100 μm. DP, dermal papilla; qPCR, quantitative polymerase chain reaction.

was performed for PRP diluted at 5% in saline ($n = 3$; See **Supplemental Digital Content 3**, Figure S2, http://links.lww.com/DSS/A139). Similar expression patterns were found for DP markers, mitogenic factors, and hair germ quiescence markers when compared to undiluted PRP. However, regarding the inflammatory markers, dilution of PRP rescued the significant increase observed for *IL6* in undiluted PRP and decreased the expression levels of *STAT3* (0.15-fold, $p < .05$), suggesting that it is better to use 5% PRP. Additionally, there was significant ($p < .01$) 0.31-fold decrease in *p21*. Surprisingly, trypan blue staining was apparently indistinct between saline and 5% PRP stored grafts ($n = 3$), indicating that concentrated PRP may block trypan blue staining due to plasma gelification rather than by truly preventing apoptosis.

Impact of ATPv-Supplemented Saline on Hair Grafts Gene Signature and Viability

Finally, to tackle the problem of ischemic storage, ATPv-supplemented saline was tested. Similarly to saline storage alone, increased gene expression was observed for *αSMA* (0.57-fold, $p < .05$) and *KI67* (0.26-fold, $p < .0001$), again suggesting a proliferative status of hair matrix and ORS cells in these conditions. Furthermore, *VCAN* marker (1.55-fold, $p < .01$) and *FGF7*, *TGFβ*, and *IGF1R* mitogenic factors (*FGF7*, 0.52-fold, $p < .05$; *TGFβ*, 0.43-fold, $p < .01$; *IGF1R*, 0.11-fold, $p < .05$) were found concomitantly increased in ATPv-supplemented saline, suggesting that ATPv is beneficial for hair growth and anagen maintenance when compared to saline solution alone. Importantly, no significant changes were found for trichogenic markers or genes involved in hair germ quiescence and inflammation (Figure 5A–E). Moreover, ATPv-supplemented Hypothermosol also revealed beneficial when compared to Hypothermosol alone (See **Supplemental Digital Content 4**, Figure S3, http://links.lww.com/DSS/A140), even though to less extent than ATPv-supplemented saline (Table 1). Although trypan blue staining did not suggest clear improvement in hair graft viability for ATPv-supplemented saline stored grafts (Figure 5F), gene expression data indicated that ATPv is more effective for hair grafts storage at 4°C.

Discussion

Several conditions are currently used in the clinics to store FUE micrografts ex vivo but without

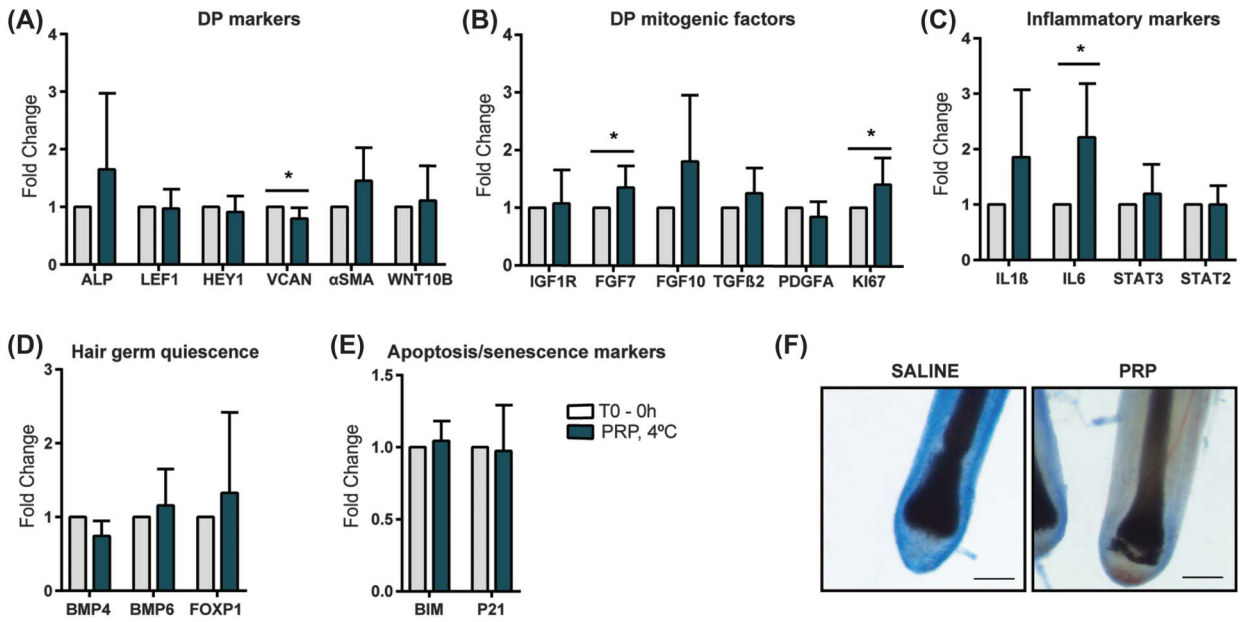

Figure 4. Impact of PRP storage on gene expression signature of hair follicle bulbs. Micrografts from 7 patients were stored in autologous PRP at 4°C for 6 hours. (A–E) Gene expression analysis of DP marker genes, as well as of DP mitogenic signaling, pro-inflammation, hair germ quiescence, and apoptosis/senescence. Gene expression was measured by qPCR after 6 hours of storage, and fold change was compared to freshly isolated hair bulbs (T0 − 0 hour). Data are presented as mean ± SD.*$p < .05$ by Student unpaired *t*-test statistical analysis. (F) Hair grafts viability after 6 hours of storage in activated PRP. Viability is inversely correlated with the blue staining. Saline stored grafts were used as control. Scale bars, 100 μm. DP, dermal papilla; PRP, platelet-rich plasma; qPCR, quantitative polymerase chain reaction.

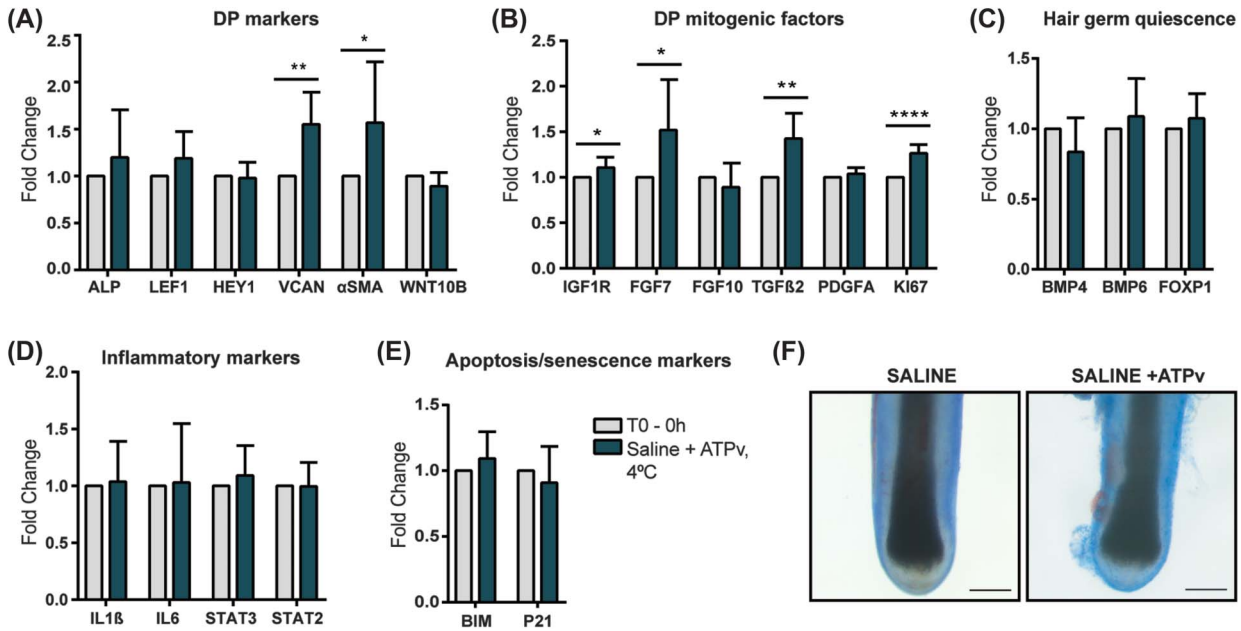

Figure 5. Impact of ATPv-supplemented saline on gene expression signature of hair follicle bulbs. Micrografts from 7 patients were stored in ATPv-supplemented saline at 4°C for 6 hours. (A–E) Gene expression analysis of DP marker genes, as well as of DP mitogenic signaling, hair germ quiescence, pro-inflammation, and apoptosis/senescence. Gene expression was measured by qPCR after 6 hours of storage, and fold change was compared to freshly isolated hair bulbs (T0 − 0 hour). Data are presented as mean ± SD. *$p < .05$, **$p < .01$, ****$p < .0001$ by Student unpaired *t*-test statistical analysis. (F) Hair grafts viability after 6 hours of storage in ATPv-supplemented saline. Viability inversely correlates with the blue staining. Saline stored grafts were used as control. Scale bars, 100 μm. DP, dermal papilla; qPCR, quantitative polymerase chain reaction.

demonstrated efficacy.[1] The authors postulated that exposing hair grafts to different environments may distinctly affect signaling pathways controlling hair growth and cycling, mainly regulated by DP. Stresses arising from hair graft storage might result in transient postsurgical hair shedding by triggering premature entry into catagen or compromising viability.[1,23]

The findings of this study demonstrate that distinct storage conditions differently impact on gene expression of key signaling molecules involved in hair cycle. In particular, the authors assessed genes of a molecular signature enriched in intact DP, which has been reported to promote anagen and hair growth.[20]

The authors also found that storage temperature plays a relevant role in grafts' viability and inflammation, as RT leads to upregulation of *BIM* and *IL1β* markers, particularly in DPC medium. Of note, *IGF1R* expression is also decreased for grafts stored in saline at RT. Considering the role of *IGF1/IGF1R* in hair growth,[19] a decrease in *IGF1R* together with an increase in *BIM* and *IL1β*, suggests potential induction of anagen-to-catagen transition for grafts stored at RT. The results of this study are in agreement with previous studies showing that cold storage improves graft survival[24] without significantly altering the morphological structure of HF.[5] Next, the authors tested the best biochemical environments for hair graft preservation under cold temperature. Examination of micrografts stored in chilled isotonic solutions (saline and DPC medium) evidenced that those solutions partially preserve hair grafts' quality. However, both solutions induced *STAT2* expression, a mediator of the *JAK-STAT* inflammatory signaling pathway.[25] Also, the authors found significant decrease in *HEY1* levels in chilled saline stored grafts. *HEY1* is required for follicle maintenance through *β-catenin*–induced activation of DP cells.[26,27] Considering that *JAK-STAT* signaling inhibits anagen and hair growth,[20] data of the present study suggest that chilled isotonic storage may drive anagen-to-catagen transition. In agreement, an increase was observed for *FOXP1* levels for saline stored grafts. *FOXP1* is essential for maintaining HFSC quiescence[28] and is responsive to reactive oxygen species (ROS), controlling HFSC proliferation during anagen.[29] Hence, the study data also suggest that chilled saline storage is compromising HFSC activation, possibly due to an increase in ROS. In contrast, HypoThermosol storage prevented increased levels of inflammatory markers (*IL6* and *STAT2*), as well as of the cyclin-dependent kinase inhibitor *p21*. Overexpression of both *IL6* and *p21* are known to inhibit hair growth.[30,31] Thus, the study data corroborates that HypoThermosol mitigates the cellular stress caused by chilling. Nevertheless, similarly to saline, significant decrease was observed for *HEY1* DP marker. Moreover, the expression of *WNT10B* and *PDGFA* was strongly repressed. *WNT10B*, a *Wnt* signaling molecule upstream of *LEF1*, is solely expressed during anagen and it is involved in HF induction ability and growth promotion of DP.[32] Transcriptional repression of *PDGFA*, a mitogenic factor crucial for the induction of HF regeneration, was also observed.[33] Together, these findings strongly suggest that, even though HypoThermosol protects tissue from storage hypothermic stress, it does not preserve anagen gene signature (Figure 6).

Platelet-rich plasma contains numerous nutrients and growth factors that may prove advantageous for hair graft growth and viability.[34] Increased expression of *FGF7* and *KI67* proliferation markers and concomitant decrease in *BMP4* were observed in chilled PRP storage (Figure 6). These results are in agreement with previous studies reporting that activated autologous PRP induces DP cell proliferation by upregulating *FGF7* and *β-catenin*.[8] *BMP4*, expressed in hair matrix and DP,[35] is known to prevent anagen onset and suppress hair growth by inhibiting *Wnt* and *Shh* signaling.[36] Concomitant with the gene expression data, PRP stored grafts are clearly more viable than saline stored grafts by macroscopic analysis. Hence, these observations indicate an anagen retaining effect of PRP. Surprisingly, *IL6* is clearly increased in PRP storage. *IL6* upregulation was reported to inhibit hair shaft elongation and matrix cells' proliferation[37] and to trigger anagen-to-catagen transition.[31] Ambiguously, *IL6* was shown to promote wound-induced HF neogenesis through *IL6/STAT3* signaling pathway[38] and to promote hair growth in mice.[39] Given the decrease in *VCAN* expression, an anagen DP marker implicated in hair regeneration,[40] these data suggest that the inflammatory response may be counteracting

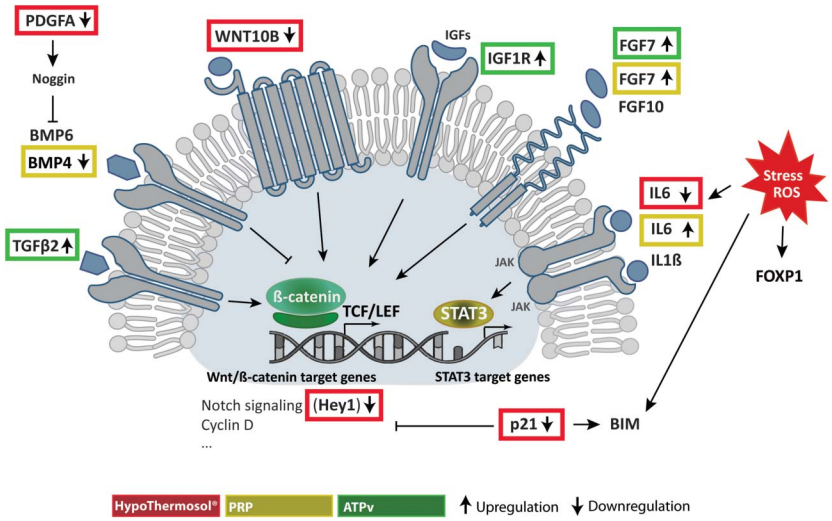

Figure 6. Graphical summary of the impact of HypoThermosol, PRP, and ATPv-supplemented saline hair graft storage in the major signaling pathways of the feedback regulatory loop between DP and HFSC. Dermal papilla regulates several intercellular signaling pathways, including TGFβ, PDGF, WNT, IGF, and FGF, which mediate the instructive signals into HFSC activation through β-catenin-*TCF/LEF1* transcriptional activation. External stress pathways induce JAK/STAT signaling, which counteracts the instructive signals for hair regeneration, by compromising epithelial–mesenchymal interactions between HFSC and DP. Genes whose expression was found upregulated and/or downregulated in hair follicle bulbs of grafts stored in HypoThermosol, PRP, or ATPv-supplemented saline are highlighted in red, yellow, and green boxes, respectively. DP, dermal papilla; HFSC, hair follicle stem cell; PRP, platelet-rich plasma.

the anagen-promoting effect of *FGF7*. Finally, inflammatory phenotype suppression was clearly evident for grafts stored in ATPv-supplemented saline. Importantly, the study results also show that ATPv-supplemented saline enhances HF survival and triggers activation of growth factor signaling pathways involved in anagen sustained growth (*IGF1R*, *FGF7*, and *TGFβ2*) (Figure 6). *TGFβ2* is specifically expressed in human DP cells[41] and is recently suggested as a major pathway regulating adult hair cycle by antagonizing BMP signaling and prompting tissue regeneration.[42] The *VCAN* anagen marker and *KI67* proliferation marker were also significantly increased. Increased levels of growth signaling pathways were reported for HF anagen growth phase in vivo.[10] Therefore, the authors postulate that a pronounced upregulation of mitogenic signals in ATPv-supplemented saline may sustain DP cells survival, preventing catagen onset. Ultimately, neither DP biomarkers (*ALP* nor *LEF1*) nor *BIM* pro-apoptotic marker was affected after 6 hours of chilled storage, evidencing this time frame to appropriately maintain DP identity of HF ex vivo.

Overall, the study data suggest chilled ATPv-supplemented saline as the better solution for storage of HF grafts, as it better preserves the in vivo gene expression anagen profiling (Figure 6). This is in agreement with previous clinical findings demonstrating improved growth of hair micrograft transplants after ATPv-supplemented Hypothermosol versus Hypothermosol alone,[9,43] suggesting that ATPv, rather than Hypothermosol, contributes primarily to that clinical outcome. Interestingly, the authors found that ATPv-supplemented saline was nevertheless more beneficial than ATPv-supplemented Hypothermosol in the maintenance of the intact DP molecular signature. Trichoscopy monitoring should be used to further validate the impact of this storage solution on postsurgical hair growth. Even so, the gene expression profiling here described will be extremely useful for testing other existing holding solutions, as well as for understanding the mechanisms prompting apoptosis and catagen during grafts' storage, thus contributing to clinical improvement of hair restoration surgery.

Acknowledgments The authors thank members of the Saúde Viável–Clínica de Microtransplante Capilar and Aging and Aneuploidy group (i3S) for their assistance. E. Logarinho holds an FCT Investigator Postdoctoral Grant (IF/00916/2014) from

FCT/MCTES (Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior). Saúde Viável and North Regional Operational Program (NORTE2020) under PORTUGAL 2020 Partnership Agreement through Regional Development Fund (FEDER) supported this work.

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