Article

Plastic Surgery
2017, Vol. 25(1) 40-47
© 2017 The Author(s)
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/2292550317693819
journals.sagepub.com/home/psg

The Use of Platelet-Rich Plasma for Storage of Surplus Harvested Skin Grafts

L'utilisation de plasma riche en plaquettes pour conserver des greffons de peau excédentaires

Ilknur Keskin, MD, PhD[1,2], Nilufer Ayturk, MSc[1,2], Mustafa Sutcu, MD[3], Mustafa Keskin, MD[3], and Tangul Mudok, PhD[1,2]

Abstract

Background: There is a need for improved methods and storage media to sustain the tissue viability of autologous skin grafts. **Objective:** To compare histological changes in human skin grafts stored in platelet-rich plasma (PRP) with those of grafts stored in saline. **Methods:** Eight circular, 3-mm full-thickness skin graft samples were harvested from the abdominal skin of each of 5 patients scheduled to undergo an abdominoplasty procedure. Four of these graft samples were stored in saline, and the other 4 were stored in saline mixed with PRP prepared from the patient's own venous blood. Histological assessment of the microscopic appearance of the samples was performed on days 5, 8, 11, and 14. The integrity of the epidermal–dermal junction, number of keratinocytes with perinuclear halos, collagen organization, and number of fibroblasts per field were assessed. The cellular apoptosis rate was also measured on these same days. **Results:** On day 5, significant differences were observed microscopically between the PRP- and saline-stored grafts ($P < .05$). The grafts preserved in saline exhibited early marked cellular and nuclear swelling with pleomorphism, as well as early nuclear halo formation. The cell viability rate of the PRP group was significantly higher than that of the saline-stored group on day 8 ($P < .05$). **Conclusion:** Platelet-rich plasma and its inherent growth factors supported longer graft survival; however, its effect lasted only until day 8. Platelet-rich plasma may be beneficial if grafts need to be stored for delayed application(s).

Résumé

Historique : Il faut améliorer les méthodes et les milieux de conservation pour maintenir la viabilité des greffons autologues de peau. **Objectif :** Comparer les changements histologiques des greffons de peau humaine conservés dans du plasma riche en plaquettes (PRP) à ceux des greffons conservés dans un soluté physiologique. **Méthodologie :** Les chercheurs ont prélevé huit échantillons de greffons circulaires de trois millimètres de peau pleine épaisseur sur la peau de l'abdomen de chacun des cinq patients qui devaient subir une abdominoplastie. Quatre d'entre eux ont été conservés dans un soluté physiologique et les quatre autres, dans un soluté physiologique mélangé à du PRP préparé à partir du propre sang veineux du patient. Les chercheurs ont procédé à l'évaluation histologique de l'aspect microscopique des échantillons les cinquième, huitième, onzième et quatorzième jours après le prélèvement. Ils ont examiné l'intégrité de la jonction dermoépidermique, le nombre de kératinocytes dotés de halos périnucléaires, l'organisation du collagène et le nombre de fibroblastes par champ. Les mêmes jours, ils ont mesuré le taux d'apoptose cellulaire. **Résultats :** Le cinquième jour, les chercheurs ont observé des différences microscopiques significatives entre les greffons conservés dans le PRP et ceux conservés dans un soluté physiologique ($P<0{,}05$). Les greffons conservés dans le soluté physiologique présentaient une hypertrophie cellulaire et nucléaire précoce marquée

[1] Department of Histology and Embryology, Istanbul Medipol University, Istanbul, Turkey
[2] Regenerative and Restorative Medical Research Center (REMER), Istanbul Medipol University, Istanbul, Turkey
[3] Department of Plastic and Reconstructive Surgery, Istanbul Medipol University, Istanbul, Turkey

Corresponding Author:
Mustafa Keskin, Department of Plastic and Reconstructive Surgery, Istanbul Medipol University Medical School, TEM Avrupa Otoyolu, Bagcilar, Istanbul 34200, Turkey.
Email: mkeskin@medipol.edu.tr

accompagnée de pléomorphisme ainsi que la formation précoce de halos nucléaires. Le huitième jour, le taux de viabilité des cellules du groupe de PRP était considérablement plus élevé que celui des cellules du groupe de soluté physiologique ($P<0,05$). **Conclusion :** Le plasma riche en plaquettes et les facteurs de croissance inhérents favorisaient une plus longue survie, mais seulement jusqu'au huitième jour. Le plasma riche en plaquettes peut être bénéfique si les greffes doivent être conservées en vue d'être utilisées plus tard.

Keywords
graft storage, graft survival, platelet-rich plasma, tissue viability

Introduction

Autologous skin grafts continue to be the major source for covering skin defects. In some clinical circumstances, however, not all of the harvested skin grafts may be used during surgery. In such cases, the unused skin should be stored for possible use in future application(s).

For graft storage to be successful, the structural integrity and viability of the graft must be maintained until it is transferred to the recipient bed. The storage solution and temperature affect the viability of the stored graft.[1,2] There are several available methods for graft storage. The standard method is to store the skin in normal sterile saline-soaked gauze at 4°C. This method is simple, effective, readily available, and cost-effective. However, the search for an ideal solution—one that is electrolyte- and nutrient-rich—to sustain graft tissue viability during storage continues.

Platelet-rich plasma (PRP) represents the portion of the plasma fraction of autologous blood with a platelet concentration above baseline values.[3] Platelet-rich plasma contains >30 bioactive proteins, many of which play a fundamental role in tissue healing. Platelet-rich plasma also includes 3 proteins known to act as cell-adhesion molecules—fibrin, fibronectin, and vitronectin.[4] Its clinical use has surged due to its potential healing properties through the recruitment, proliferation, and differentiation of cells and tissue remodelling[5] and has gained popularity in almost all fields of surgery. Blood is drawn from the peripheral vein and subsequently stored with an anticoagulant, such as acid citrate dextrose solution A. To separate the components of blood according to specific weight and to increase the concentration of platelets, whole blood is subjected to centrifugation. The resulting pellet contains a platelet concentration above the baseline level of whole blood.

In the present study, we hypothesized that PRP would be a favourable storage medium for skin grafts. Accordingly, we compared the histological changes in human skin grafts stored in PRP to those of grafts stored in saline.

Methods

Patients and Graft Harvesting

After obtaining informed consent and written regulatory approval in accordance with the institutional review board, venous blood was drawn from 5 patients scheduled to undergo abdominoplasty. Full-thickness skin was harvested from the abdominal area, which was to be excised and discarded during the abdominoplasty procedures. Using a 3-mm punch biopsy, 8 circular 3-mm full-thickness skin graft samples were harvested. Care was taken to select an area away from the abdominal striae. The patients did not have any systemic illness or skin pathology. The skin graft samples were placed in sterile specimen containers containing a storage solution. The containers were then divided into 2 groups of 4 containers—4 containing saline only (0.9% sodium chloride [10 mL ampule], Adeka, Turkey) and 4 containing saline and PRP. The volume of the storage solution was 5 mL. All samples were stored in a temperature-monitored refrigerator with set point of 4°C (temperature maintained between 2°C and 8°C). The storage solution was not refreshed throughout the time course of the study.

Storage and Handling of PRP

The preparation of PRP was highly standardized, and the same procedure was used with each of the 5 patients. An APC-60 Harvest PRP procedure pack with the Smart PRep 2 system (Harvest Terumo BCT, Lakewood, CO, USA) was used to produce the PRP according to the method recommended by the manufacturer. Blood (52 mL) was drawn into adenosine citrate-dextrose acid containing 60 mL syringes. The Smart PReP 2 kit uses an automated dual spin centrifugation process to separate PRP from whole blood. The total volume is then transferred to a disposable, dual-chamber blood-processing device, which is then placed in a centrifuge. During centrifugation, the blood is separated into 2 distinct chambers—one containing packed red blood cells, and the other containing autologous platelet concentrate and plasma supernatant. Whole blood from each patient was processed using the system according to the manufacturer's instructions, which produced 10 mL of PRP as the end product.

Group Allocation

Group 1 (PRP group)—4 sterile, sealable specimen jars were prepared, and a 3-mm skin graft sample was placed in each of the jars containing 2.5 mL of saline plus 2.5 mL of PRP for each patient (total of 20 specimens/5 patients). Group 2 (saline group)—4 sterile, sealable specimen jars were prepared, and a 3-mm skin graft sample was placed in each of the jars containing 5 mL of saline (total of 20 specimens/5 patients).

Histopathological Evaluation of Tissue Integrity

The skin tissues were fixed in 4% formalin and subsequently embedded in paraffin according to standard procedures.

Table 1. Comparison of the Microscopic Features of Skin Grafts Stored in Saline- and Platelet-Rich Plasma (PRP).[a]

	Day 5		Day 8		Day 11		Day 14	
Features	Saline	PRP	Saline	PRP	Saline	PRP	Saline	PRP
Integrity of epidermal–dermal junction	1.4	2	0.6	1.6	0.6	1	0	0.4
Perinuclear halo of keratinocytes	1.6	1.8	0.8	1.8	0.6	1.2	0.2	0.4
Collagen organization	2	2	1.6	1.8	1	1.2	0.6	0.4
Presence of fibroblasts	1.6	2	1	2	1	1.2	0.4	1
Total	6.6	7.8	4	7.2	3.2	4.6	1.2	2.2
P	.005		.001		.058		.046	

[a]Data presented as mean scores (see "Histopathological Evaluation of Tissue Integrity" for scoring details) unless otherwise indicated.

Sections (4 μm thick) were cut using an electronic rotary microtome (Microm HM 340E; Thermo Scientific, Germany). Sections were stained with Masson's trichrome included in a commercially available blue aniline staining kit (04-010802, Bio-Optica, Milano, Italy) for qualitative examination of collagen and elastin fibres, in addition to staining with hematoxylin–eosin for morphological analysis. Histological assessments were performed on days 5, 8, 11, and 14; microscopic features of the samples were assessed in a blinded fashion using a microscope (Axio Zoom v16; Carl Zeiss, Germany). The microscope samples were evaluated, and histological skin damage among the groups was compared by scoring the severity of the damage according to the method of Turhan-Haktanir et al.[6] Four different features were rated using a 3-point scale, with a higher score representing less structural disruption of skin layers. The first feature evaluated was the integrity of the epidermal–dermal junction; the second was the formation of perinuclear halos of keratinocytes, with 2 points assigned for a rate <25%, 1 point assigned for a rate of between 25% and 50%, and 0 points given for a rate >50%; the third feature was collagen organization, with 2 points given to grafts with normal collagen organization, 1 point given for disturbed organization, and 0 points given for amorphic organization; and the final parameter was the number of fibroblasts per field under 400× magnification. Grafts with >10 fibroblasts were assigned 2 points, those with between 5 and 10 received 1 point, and those with <5 received 0 points.

Determination of the Rate of Cellular Apoptosis

Apoptosis was assessed by in situ terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling (TUNEL) using a commercially available kit (In situ Cell Death Detection kit, Fluorescein, version 17; Roche Diagnostics, Germany). Poly-L-lysine-coated slides and a Zeiss LSM 780 NLO multiphoton confocal microscope (Carl Zeiss, Germany) were used. The investigators were blinded to the grouping. Nonoverlapping slices were photographed continuously from left to right and up and down. The photographs were numbered, and random fields were obtained using a random number method generated by a computer. The apoptotic rate was determined by counting the number of TUNEL-positive cells among the visible cells at a magnification of 20×, as per the method of Knapik et al.[7] The mean value of at least 5 counts was calculated; the final values were subjected to statistical analysis.

Statistical Analysis

SPSS version 18.0 (SPSS© Inc, IBM, Chicago, IL, USA) was used for data analysis. Differences between values were assessed using an analysis of variance test, followed by the least significant difference test for post hoc comparisons. All data were expressed as mean (± standard error) and $P < .05$ was considered statistically significant.

Results

Abnormalities of different severities began to develop in all skin layers of all samples in both groups over time. On day 5, significant differences were observed between the PRP- and saline-stored skin grafts ($P < .05$). Compared to the saline-stored grafts, the histological scores of the grafts stored in PRP were significantly higher (Table 1). In contrast to the PRP-stored grafts, the grafts preserved in saline exhibited early marked cellular and nuclear swelling with pleomorphism, as well as early nuclear halo formation.

On day 8, there were distinct differences between the 2 groups ($P < .05$). In the PRP group, there were signs of epidermal clefting; however, complete epidermal separation was not observed in any of the grafts. After day 11, the appearance of the samples in both groups was similar, with areas of separation between the epidermis and dermis. In both groups, the dermal collagen became more uniform and less structured, with loss of differentiation between the papillary and reticular dermis. In both groups, the subepidermal capillary vessels appeared normal. By day 14, the majority of the specimens in the saline group showed clear dermoepidermal separation, whereas dermoepidermal separation was not as apparent in the PRP group (Figures 1 and 2).

The findings of TUNEL are shown in Figure 3. Apoptosis was more apparent in cells in the saline group than in the PRP group. The mean (± SE) apoptotic rates in the saline-only group were 19.8% ± 3.2% on day 5, 29.2% ± 2.7% on day 8, 31.8% ± 2.0% on day 11, and 35.8% ± 2.8% on day 14.

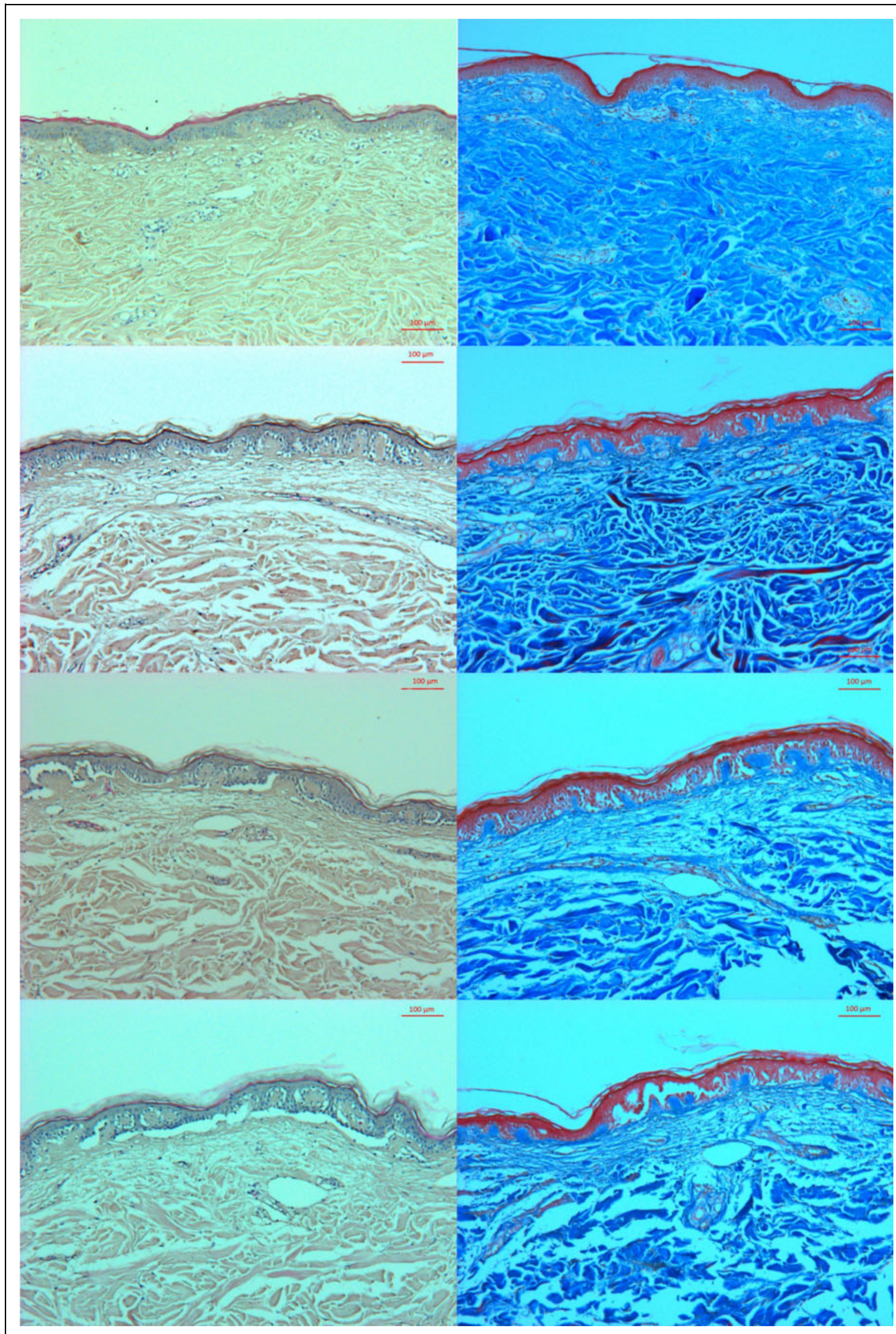

Figure 1. Microscopic characterization of skin grafts stored in saline. The left column shows the samples stained with hematoxylin and eosin, and the right column shows those stained with Masson's trichrome. From the upper row to the bottom, the figures are from days 5, 8, 11, and 14, respectively.

The mean apoptotic rates in the PRP group were 20.0% $\pm$ 3.0% on day 5, 23.8% $\pm$ 1.8% on day 8, 29.4% $\pm$ 1.4% on day 11, and 33.0% $\pm$ 2.0% on day 14 (Figure 4). On day 8, the cellular apoptosis rate of the skin grafts stored in PRP was significantly lower than that of the grafts stored in saline only ($P < .005$).

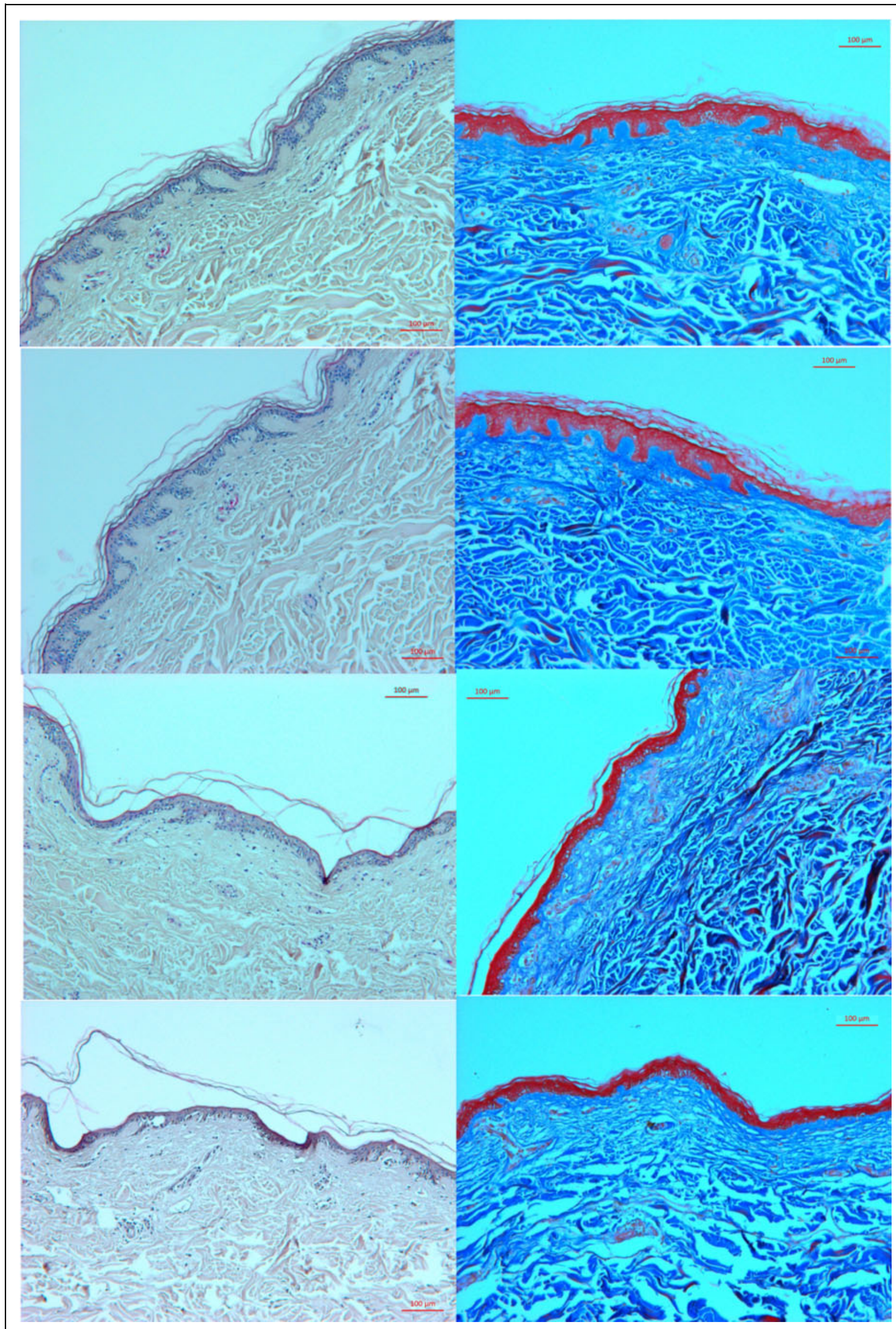

Figure 2. Microscopic characterization of skin grafts stored in platelet-rich plasma. The left column shows the samples stained with hematoxylin and eosin, and the right column shows those stained with Masson's trichrome. From the upper row to the bottom, the figures are from days 5, 8, 11, and 14, respectively.

Discussion

Physiological saline is not an ideal graft storage solution. Roswell Park Memorial Institute solution, University of Wisconsin solution, Hartmann's solution, Dulbecco's modified Eagle's medium, Marshall's solution, McCoy's 5A medium, keratinocyte nutrient MCDB 153, histidine–tryptophan–

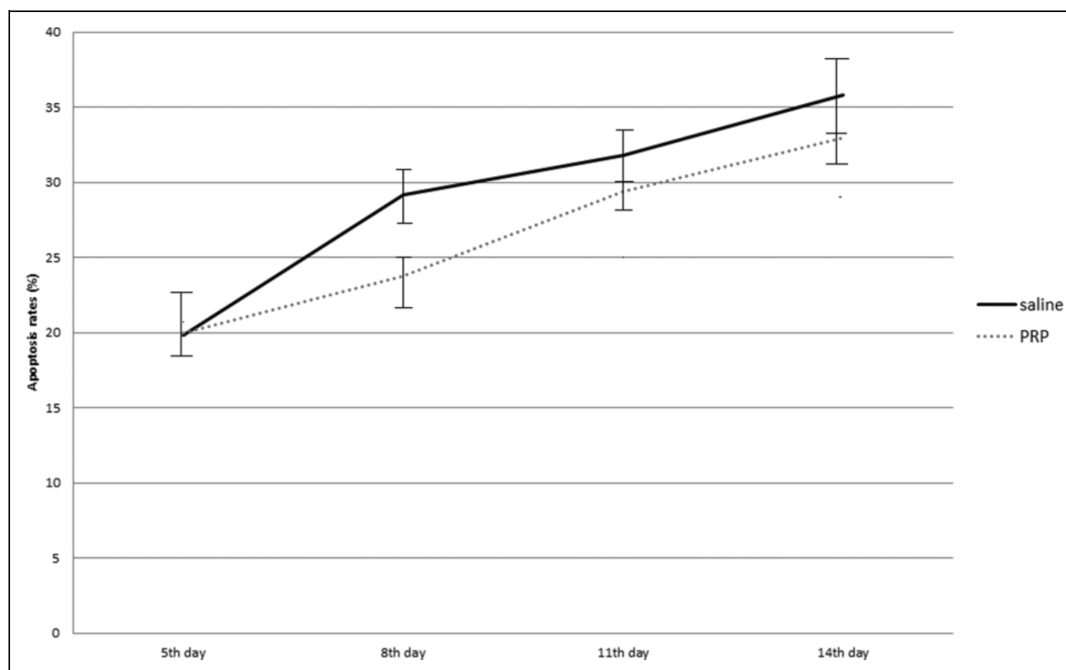

Figure 3. Results of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling (TUNEL) of cellular apoptosis for given days in samples stored in saline- and platelet-rich plasma.

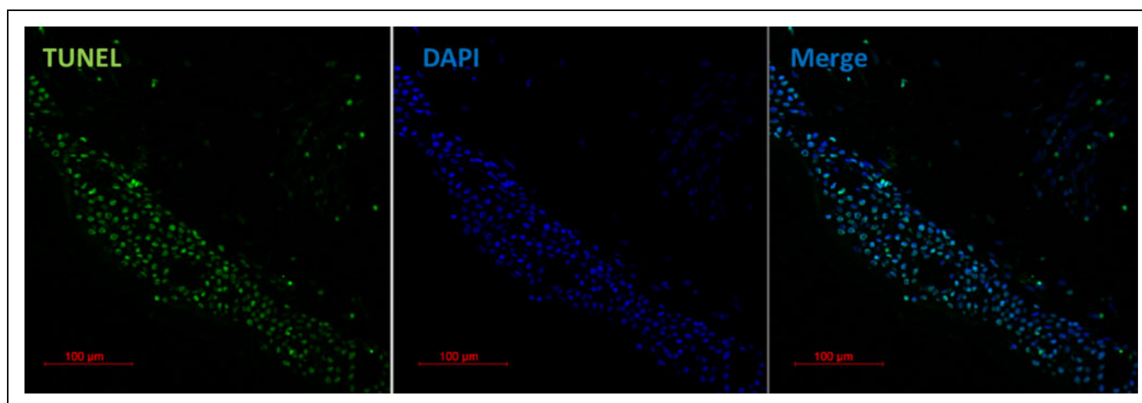

Figure 4. An example of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling (TUNEL) with significant apoptosis rate.

ketoglutarate solution, or amniotic fluid are other clinically and experimentally used storage media.[7-9] Cell culture media at 4°C are generally superior to saline in extending tissue survival time. Aside from refrigeration at 4°C in storage medium, there are 2 alternative ways to prepare and store skin samples—one is by freezing after cryopreservation with glycerol; the other is storage at room temperature after freeze-drying.[10] Both freezing and freeze-drying, however, require special equipment and are not practical in a clinical setting.

In general, studies have reported a gradual reduction in the viability of tissue over time under all storage conditions.[1,2,6,7] There is some evidence that storing skin samples at 4°C for several weeks can lead to structural and metabolic degradation of skin cells. Following harvesting, keratinocytes stored at room temperature die over several days due to an accumulation of toxic metabolites and a lack of nutrition.[11,12] Storing skin grafts at 4°C prolongs their viability by attenuating cellular metabolism without risking ice crystal formation.[1] A previous study reported that storing skin grafts at 4°C in saline for >4 weeks preserved >50% of keratinocyte viability.[13] In other studies, however, skin grafts stored under similar conditions lost approximately 90% of their viability after 10 days.[14,15] Therefore, there is a need to develop methods to maximize the viability of skin samples/grafts stored in refrigerators.

Cetin et al[16] initially proposed using human plasma to store skin grafts. The viability of human skin grafts stored in plasma and those stored in saline at 4°C was compared by assessing the number of viable keratinocytes using the trypan blue method. The percentage of viable keratinocytes was higher in skin

grafts stored in plasma after 30 days; the authors concluded that plasma was a better storage medium. They attributed their findings to the fact that saline containing electrolytes only cause saline-stored skin grafts to lose some of their viability in a short period and become edematous. Although plasma may provide a better milieu for skin grafts, the authors warned that plasma-containing media may promote bacterial growth, and the plasma-stored skin grafts should not be used as homografts because of the risk of blood-borne diseases.

The results of the present study showed that PRP increased the quality and survival time of stored skin grafts. The superior performance of PRP compared to saline only is believed to be due to its composition, given that it is a natural source of growth factors. In platelets, cytokines and growth factors are stored in α-granules in their incomplete form. Of these, platelet-derived growth factor, transforming growth factor-β 1 (TGF-β1), vascular endothelial growth factor, and epidermal growth factor are considered the most important.[17] Platelet-rich plasma plays a critical role in the repair and the regeneration of different tissues via the activation and secretion of these growth factors and other cytokines stored in the α-granules of platelets. The concentration of platelets in PRP is important, with 1×10^6 platelets/μL suggested as the minimal therapeutic level.[18] Platelet-rich plasma varies in composition according to its mode of preparation. Platelet-rich plasma needs to be activated to enable the α-granule membrane to fuse with the platelet membrane and secretory proteins, including growth factors, to become bioactive.

To date, conclusive evidence supports the use of PRP to improve the healing of diabetic lower-limb ulcers, enhance bone grafting, and increase the survival rate of fat grafts.[19] The actions of mitogenic and chemotactic growth factors are believed to increase the viability of grafts by impeding degenerative events that lead to the formation of cysts, vacuoles, and fibroses.[20] Platelets express chemokine receptors, which regulate inflammatory responses and prevent excess leukocyte recruitment by anti-inflammatory cytokines such as TGF-β.[20] In an in vitro study,[21] PRP suppressed enzymes, such as cyclooxygenase-1, cyclooxygenase-2, and membrane prostaglandin E synthase, which are active in the inflammatory pathway, suggesting that PRP has an anti-inflammatory effect. Local PRP application also appeared to have an inhibitory effect on specific proinflammatory cytokines, specifically through suppression of interleukin 1 release from activated macrophages.[22]

Studies investigating PRP share some common drawbacks. Although many different protocols have been used for its preparation, it has been difficult to standardize a protocol. Various platelet separation processors are commercially available, and centrifugation speeds and times are also different. These result in differences in the concentrations of platelets, leukocytes, red blood cells, and growth factors. All of these variables may affect the host's cellular response. Another point to consider is the timing of the release of growth factors or cytokines after the storage process has begun. Studies have found that >95% of presynthesized growth factors were secreted within the first hour[23] and that they continued to be synthesized until day 8.[24] Several studies have confirmed the gradual release of platelet-derived growth factors and TGF for up to 28 days.[23-25]

Several authors have previously reported detailed histological changes in human skin grafts stored at 4°C in various configurations over a 4-week period.[1,2,8,10-15] Cellular and nuclear swelling with pleomorphism have been reported to be early changes in stored grafts; nevertheless, grafts at this stage are believed to be viable. The formation of blebs that lead to epidermal separation is an important indicator of viable keratinocytes. In our study, the most pronounced differences between the PRP and saline groups were observed on day 8, with the saline-stored skin grafts showing a severe degree of dermoepidermal clefting. Similar clefting was not apparent in the PRP-stored skin grafts until after day 14.

There are many ways of preparing PRP; currently, however, approximately 60 mL of venous blood will yield only 10 mL of PRP as an end product. For this reason, it does not appear to be clinically feasible to use only pure PRP in clinical settings, where significant volumes of storage medium would be needed to store large skin grafts. Therefore, PRP should be used only as a supplement to physiological saline. For PRP to be used more routinely in storing skin grafts, its production would need to be more cost-effective, which in turn would make it more accessible. Although there are many commercially marketed systems that facilitate the preparation of ready-to-apply platelet-rich suspensions in a reproducible manner, the high costs of these PRP processing kits remain prohibitive.

Conclusion

Platelet-rich plasma is believed to be a useful storage medium due to the increased concentration of essential cytokines and growth factors released by activated platelets. The aim of the current study was to investigate the feasibility of PRP as a storage material for skin grafts. We hypothesized that this nutrient-rich material and its wide variety of growth factors may help to increase the survival time of stored grafts. The plasma component of PRP may also provide nutrient support to the graft. Unlike other nutrient-rich media, PRP is readily available in most hospitals.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Li Z, Overend C, Maitz P, Kennedy P. Quality evaluation of meshed split-thickness skin grafts stored at 4°C in isotonic solutions and nutrient media by cell cultures. *Burns*. 2012;38(6): 899-907.

2. Peled IJ, Notea E, Lindenbaum E. Prolonged skin graft preservation with keratinocyte culture medium. *Eur J Plast Surg*. 1991; 14(5):232-234.
3. Marx R, Carlson E, Eichstaedt R, Schimmele S, Strauss J, Georgeff K. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85(6):638-646.
4. Dhurat R, Sukesh MS. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. *J Cutan Aesthet Surg*. 2014;7(4):189-197.
5. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost*. 2004;91(1):4-15.
6. Turhan-Haktanır N, Sahin O, Yagmurca M, Koken G, Demir Y, Cosar E. Histological assessment of skin grafts stored in amniotic fluid and saline. *J Plast Surg Hand Surg*. 2010;44(4-5): 226-230.
7. Knapik A, Kornmann K, Kerl K, et al. Practice of split-thickness skin graft storage and histological assessment of tissue quality. *J Plast Reconstr Aesthet Surg*. 2013;66(6):827-834.
8. Hurst LN, Brown DH, Murray KA. Prolonged life and improved quality for stored skin grafts. *Plast Reconstr Surg*. 1984;73(1): 105-110.
9. Tosun Z, Aktan TM, Hosnuter M, Savaci N, Duman S. Investigation of skin graft viability with DMEM and Ham's F-12 fluids. *Ann Plast Surg*. 2001;47(2):217-218.
10. Webster JP. Refrigerated skin grafts. *Ann Surg*. 1944;120(4): 431-448.
11. Knapik A, Kornmann K, Kerl K, et al. In vivo evaluation of wound bed reaction and graft performance after cold skin graft storage: new targets for skin tissue engineering. *J Burn Care Res*. 2014;35(4):e187-e196.
12. Sterne GD, Titley OG, Christie JL. A qualitative histological assessment of various storage conditions on short term preservation of human split skin grafts. *Br J Plast Surg*. 2000;53(4): 331-336.
13. Basaran O, Ozdemir H, Kut A, et al. Effects of different preservation solutions on skin graft epidermal cell viability and graft performance in a rat model. *Burns*. 2006;32(4): 423-429.
14. Bujía J, Osete JM, Medina A, Sprekelsen C, Wilmes E, Hammer C. Prolonged vital cartilage graft preservation using tissue culture methods. *Eur J Plast Surg*. 1994;17(1):20-22.
15. Fahmy FS, Navsaria HA, Frame JD, Jones CR, Leigh IM. Skin graft storage and keratinocyte viability. *Br J Plast Surg*. 1993; 46(4):292-295.
16. Cetin C, Kose AA, Aral E, et al. The effects of saline and plasma on skin graft keratinocyte viability. *Br J Plast Surg*. 2000;53(5): 418-419.
17. Sommeling CE, Heyneman A, Hoeksema H, Verbelen J, Stillaert FB, Monstrey S. The use of platelet-rich plasma in plastic surgery: a systematic review. *J Plast Reconstr Aesthet Surg*. 2013;66(3):301-11.
18. Pietrzak W, Eppley B. Platelet rich plasma: biology and new technology. *J Craniofac Surg*. 2005;16(6):1043-1054.
19. Serra-Mestre JM, Serra-Renom JM, Martinez L, Almadori A, D'Andrea F. Platelet-rich plasma mixed-fat grafting: a reasonable prosurvival strategy for fat grafts? *Aesthetic Plast Surg*. 2014; 38(5):1041-1049.
20. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich-fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;108(5):707-713.
21. Hanci M, Karamese M, Tosun Z, Aktan TM, Duman S, Savaci N. Intra-articular platelet-rich plasma injection for the treatment of temporomandibular disorders and a comparison with arthrocentesis. *J Craniomaxillofac Surg*. 2015;43(1):162-166.
22. Woodall J Jr, Tucci M, Mishra A, Asfour A, Benghuzzi H. Cellular effects of platelet rich plasma interleukin1 release from PRP treated macrophages. *Biomed Sci Instrum*. 2008;44: 489-494.
23. Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: a review of biology and applications in plastic surgery. *Plast Reconstr Surg*. 2006;118(6):147e-159e.
24. Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg*. 2004;62(4):489-496.
25. Liu B, Tan XY, Liu YP, et al. The adjuvant use of stromal vascular fraction and platelet-rich fibrin for autologous adipose tissue transplantation. *Tissue Eng Part C Methods*. 2013;19(1): 1-14.