ORIGINAL ARTICLE

Promotional Effect of Platelet-Rich Plasma on Hair Follicle Reconstitution in vivo

Yong Miao, MD,* Ya-Bin Sun, PhD,† Xi-Jin Sun, MD, PhD,‡ Ben-Jun Du, MD, PhD,* Jin-Dou Jiang, MD,* and Zhi-Qi Hu, MD, PhD*

BACKGROUND   Platelet-rich plasma (PRP) containing various growth factors has attracted attention in various medical fields. PRP has recently been used during hair transplantation to increase hair density.

OBJECTIVE   To investigate the effects of PRP on hair follicle (HF) reconstitution.

METHODS AND MATERIALS   Freshly isolated epidermal cells and cultured dermal papilla cells (DPCs) were mixed with various concentrations of activated PRP and transferred to a grafting chamber that was implanted onto the dorsal skin of nude mice. The chambers were removed 1 week after grafting, and HF formation was monitored for 4 weeks.

RESULTS   We observed a significant difference ($p < .05$) in the number of newly formed follicles in the area of reconstituted skin (344 ± 27 with 10% PRP vs 288 ± 35 without PRP). PRP also shortened the time of hair formation significantly; the first hairs were observed in 18 ± 1 days using 10% PRP, versus 20 ± 1 days without PRP.

CONCLUSION   A considerable effect of PRP on the time of hair formation and the yield of HF reconstitution was observed in this study. Considering the limited evidence available to judge its efficacy, further studies are required to investigate the mechanism of action of PRP.

The authors have indicated no significant interest with commercial supporters.

Androgenetic alopecia is the most common type of hair loss in men, affecting at least 50% of Caucasian men by the age of 50 and up to 70% of all men later in life.[1] Current therapeutic treatment is medication or hair transplantation, which are effective only for mild—not extensive—hair loss, and both fall short of the ultimate goal of generating new hair follicles (HFs) in a bald scalp.[2,3] Therefore, novel and effective methods of treating hair loss are urgently needed. The advent of techniques to cultivate dermal papilla cells (DPCs) and competent epithelial stem cells has recently created the opportunity to reconstruct new HFs for the treatment of hair loss.

Clues for reconstituting HFs can be gleaned from what we know about normal HF morphogenesis and growth, which involves conservative patterns of inductive and permissive signaling interplay between epithelial and dermal compartments. A number of growth and differentiation factors and their receptors are expressed in developing and mature HFs, and mutations in several of them affect hair formation. Thus, genetic evidence suggests crucial roles for platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), and fibroblast growth factor (FGF) signaling in HF development and cycling.[4,5]

Departments of *Plastic and Aesthetic Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China; †Pharmacy, Nanfang Hospital, Southern Medical University, Guangzhou, China; ‡Chengdu Taigu Technology Ltd., Chengdu, China

Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma. Growth factors, including PDGF, TGF-β, VEGF, IGF-1, EGF, and FGF, are secreted from the alpha granules of concentrated platelets activated by aggregation inducers, so PRP has been used in a wide variety of surgical procedures and clinical treatments.[6–9] PRP has recently attracted attention in the field of plastic surgery and dermatology.[10,11] It has been shown to increase the yield of transplanted follicular units when applied during male-pattern baldness surgery[12] and to promote hair growth when injected subcutaneously into C57BL/6 mice.[13]

Despite these studies, no experimental study has attempted to research the effects of PRP on HF reconstruction. Thus, the aim of the present study was to verify the efficiency of PRP on HF reconstitution.

Materials and Methods

Animals

All animal experiments were performed under Southern Medical University Animal Care and Use Committee supervision. Newborn (1–3 days old) and adult (5–6 weeks) female C57BL/6J mice and nude male mice (balb/c, nu/nu; 7–9 weeks old) were purchased from Southern Medical University, Guang Zhou, China.

Preparation of Human Activated PRP

Blood was obtained from six healthy adult human volunteers who had provided informed consent. PRP was prepared using a double-spin method, as described previously.[12] Briefly, 8–15 mL of blood was added to a centrifuge tube containing anticoagulant (3.2% (w/v) trisodium citrate) and mixed to give an anticoagulant-to-blood ratio of 10:1 (v/v) (Figure 1A).

The citrate-treated blood was centrifuged at 328 g for 10 minutes (Figure 1B), and the supernatant yellow plasma (the buffy coat containing platelets and leukocytes) was aspirated using a micropipette and then centrifuged at 4,975 g for 10 minutes (Figure 1C). The supernatant was removed, leaving only 2.5 mL of concentrate, which contains a high concentration of growth factors (Figure 1D). A 1:1 (v/v) mixture of 10% (w/v) calcium chloride and 1,000 U of bovine thrombin (Sigma-Aldrich, St. Louis, MO) was prepared in advance for use as an activator. A 10:1 (v/v) mixture of PRP and the activator was incubated at room temperature for 10 minutes. After a firm blood clot had formed, the activated PRP was centrifuged for 5 minutes at 3,184 g. The supernatant was recovered and stored at −20°C.

Preparation of Adult Mouse Vibrissae DPCs

Dermal papilla cells were isolated and cultured as described previously.[14] Briefly, the dermal papillae were dissected from mouse vibrissae follicles. The mystacial pad was cut open, the skin was inverted, and follicles were removed using fine forceps. The collagen capsules surrounding the vibrissae follicles were removed to expose the follicle bases, and the dermal papillae were dissected using thin needles. Isolated dermal papillae were placed on the bottom of a cell culture dish. The cell cultures were maintained in Dulbecco's modified essential medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco), and the medium was changed every 3 days. After the cell outgrowth became subconfluent, cells were harvested using 0.25% (w/v) trypsin–ethylenediaminetetraacetic acid (EDTA; Invitrogen, Carlsbad, CA) and subcultured at a ratio of 1:3. Afterward, DPCs were maintained in DMEM supplemented with 10% (v/v) FBS. Three-passage confluent DPCs were used for HF reconstruction.

Preparation of Neonatal Epidermal cells

Preparation of epidermal cells was done using an adaptation of an earlier study.[15] Briefly, the dorsal skin of newborn C57BL/6J mice was dissected and floated on the surface of a 0.25% (w/v) dispase solution (Sigma-Aldrich) at 4°C overnight. The piece of skin was subsequently rinsed three times in
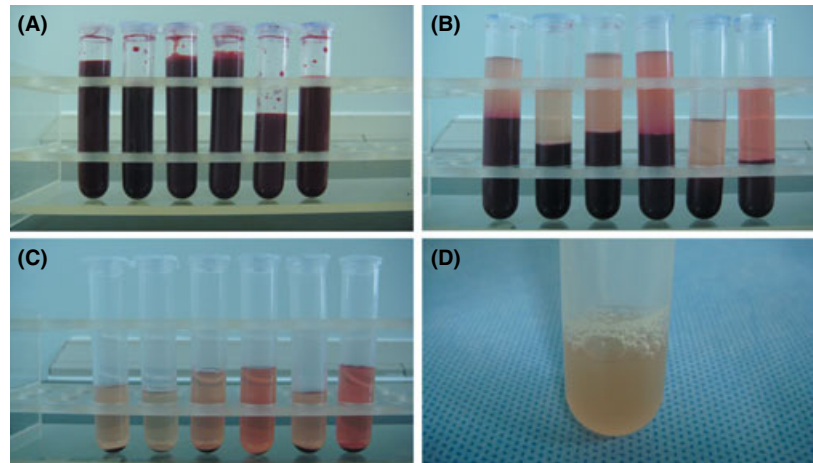
Figure 1. The preparation of platelet-rich plasma (PRP): (A) whole blood, (B) centrifuge at 328 g for 10 minutes; (C) centrifuge at 4,975 g for 10 minutes; (D) PRP.

phosphate-buffered saline (PBS; Gibco), and the epidermis was then peeled away from the dermis using forceps. The sheets of epidermis were incubated in 0.25% (w/v) trypsin–EDTA at 37°C for 1–2 hours with gentle stirring. Debris and the remaining preformed follicles were removed by passage through 100-μm-mesh-size strainers (BD Biosciences, Franklin Lakes, NJ). The cells were collected using centrifugation at 328 g for 3 minutes and suspended in DMEM for cellular grafting.

Cellular Grafting for Reconstructing HF in the Chamber Assay

The cellular grafting procedure for reconstituting HF in vivo was performed as described previously.[16] Briefly, freshly isolated epidermal cells and DPCs containing 0.5×10^6 to 12.5×10^6 cells each were suspended and mixed in 50 μL of DMEM with various concentrations of activated PRP (5%, 10%, or 15%) and transferred to a cylindrical grafting chamber (5-mm inner diameter, 5 mm high) that was implanted onto the dorsal skin of nude mice. The experiments included a normal control of cell suspensions from freshly isolated epidermal cells plus DPCs in DMEM and a negative control of epidermal cells alone. The chambers were removed 1 week after grafting, and HF formation was monitored for 4 weeks; the graft site was harvested and processed for histologic examination. The number of HFs formed in a given graft site was quantified using microscopic photography and morphometry, and three observers separately counted the HFs.

The major points of the method are summarized in Figure 2.

Statistical Analysis

All statistical analysis was done using SPSS (version 12.0, SPSS, Inc., Chicago, IL). Parametric analysis of variance with Student–Newman–Keul multiple comparison tests were used to compare groups. Statistically significant difference was set at $p < .05$. Data are presented as means $\pm$ standard deviations.

Results

Concentration of Platelets in PRP

Platelet-rich plasma prepared as described above (Figure 1) has a higher mean concentration of platelets (1.5×10^5) than whole blood (1.8×10^5 cells/μL) (Figure 3).

PRP Benefits HF Reconstitution in vivo

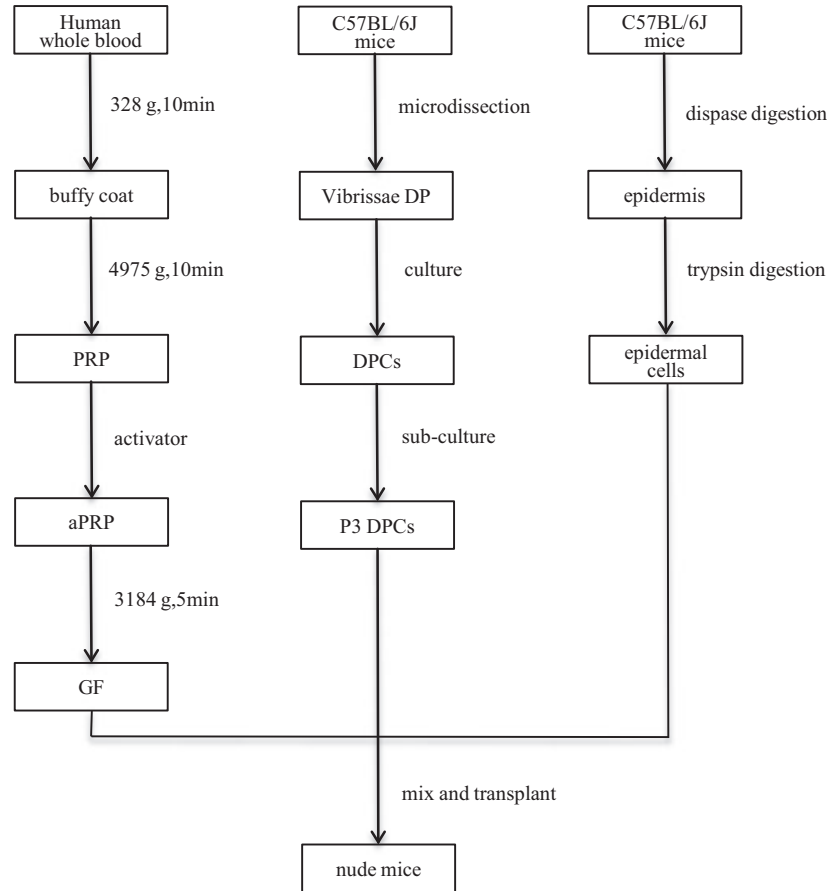The effects of PRP on HF reconstruction in vivo were assessed using a chamber assay in nude mice. Cell

Figure 2. Study diagram showing the major points of our research. DP, dermal papilla; DPCs, dermal papilla cells; GF, growth factor; PRP, platelet-rich plasma; aPRP activated platelet-rich plasma; P3, passage 3.
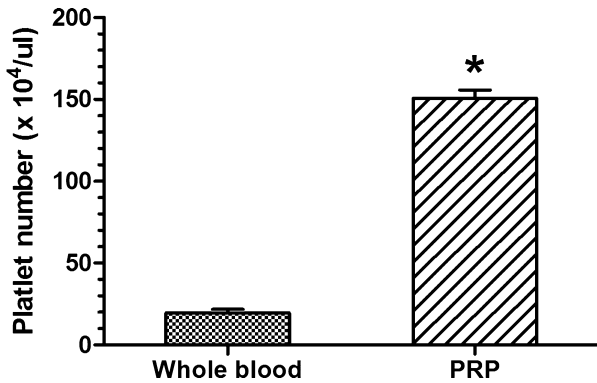

Figure 3. Concentrations of platelets after the double-spin method (n = 6). Platelet counts indicate significantly greater numbers of platelets in platelet-rich plasma than in whole blood.

grafting and PRP activation are crucial steps. In earlier studies, the total number of cells delivered and the ratio of epidermal cells to DPCs in a given chamber assay were varied according to the choice of chamber size and cell types[15–19]; we sought to optimize this relationship. The result indicates that 25×10^6 DPCs and 12.5×10^6 epidermal cells did not produce more HFs (299 ± 37) than after the transplantation of 5×10^6 DPCs and 2.5×10^6 epidermal cells (288 ± 35; Figure 4A), but when the total number of cells was reduced to 1×10^6 DPCs and 0.5×10^6 epidermal cells, significantly fewer HFs were formed (53 ± 32, Figure 4A) than the 5×10^6 DPCs. We then tested the effect of the ratio of DPCs to epidermal cells by fixing the number of DPCs at 5×10^6 and varying the number of epidermal cells from 2.5×10^6 to 12.5×10^6. A comparable number of HFs formed over this range without any significant difference (288 ± 35 and 297 ± 41, respectively), although the number of HFs formed fell to 46 ± 22 when the
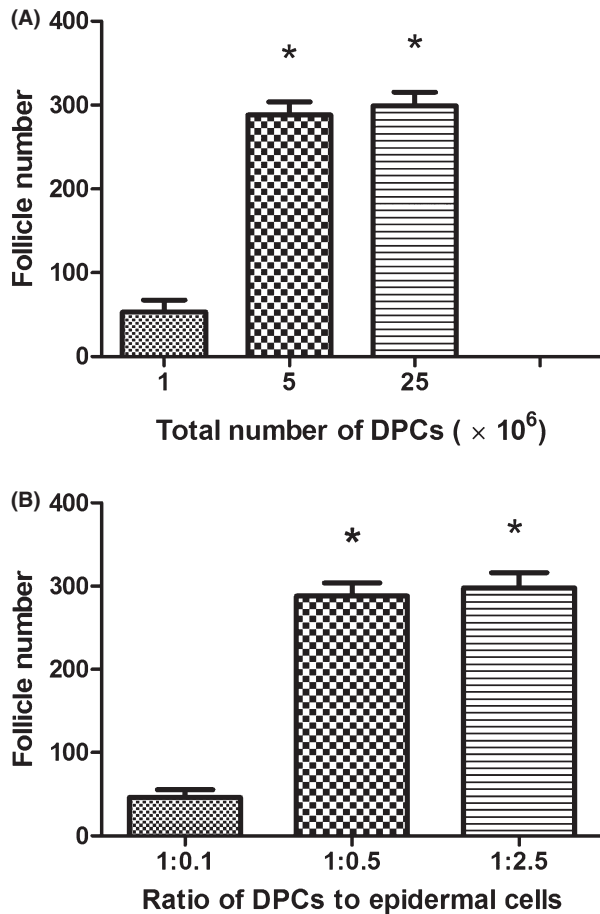
Figure 5. Effect of platelet-rich plasma (PRP) on the time of hair formation. The bar diagram shows the earliest time of forming visible hair shaft from dissociated cells. The number of dermal papilla cells was fixed at 5×10^6 for each grafting. The error bars represent standard deviations from five samples. *p < 0.05 vs the 0% activated platelet-rich plasma (aPRP) control.

Figure 4. Effect of cell number and the ratio of dermal papilla cells (DPCs) to epidermal cells on hair follicle (HF) formation. (A) Effect of total cell number grafted onto the number of HFs produced. The bar diagram shows the number of HFs generated in the chamber assay in relation to the grafted number of DPCs. The ratio of DPCs to epidermal cells was 2:1 in all cases. *p < .05 vs 1×10^6 DPCs. (B) Effect of the ratio of DPCs to epidermal cells on the number of HFs produced. The bar diagram shows the number of HFs generated in the chamber assay in relation to the ratio of the number of DPCs to epidermal cells. The number of DPCs was fixed at 5×10^6 for each grafting. The error bars represent standard deviations from five samples.*p < .05 vs DPCs. Epidermal cells = 1:0.1

cells to hair formation. The earliest time of hair formation for 10% and 15% PRP were both 18 ± 1 days, compared with 0% and 5% PRP, which were reduced significantly to 20 ± 1 days (Figures 5 and 6A).

Ten percent and 15% PRP can produce more HFs than 0% and 5% PRP. There was a statistically significant difference in the number of HFs formed using 10% (344 ± 27) or 15% (342 ± 19) PRP and 0% (288 ± 35) or 5% (297 ± 41) PRP (Figures 6B and 7).

Discussion

According to the results of this experiment, the first time of hair formation for 10% and 15% PRP (both 18 ± 1 days) is faster than for 0% and 5% PRP (both 20 ± 1 days) when grafting 5×10^6 DPCs and 2.5×10^6 epithelial cells combined with various concentrations of PRP into the chamber. The latter cases (the time of hair formation) were similar to those of earlier studies.[17–19] Nevertheless, all were slower than the time for hair shaft formation in HF embryogenesis reported earlier.[4] Although the reason

number of epidermal cells was decreased to 0.5×10^6 (Figure 4B). In all subsequent studies, each chamber assay was initiated using 5×10^6 DPCs and 2.5×10^6 epidermal cells.

When we grafted 5×10^6 DPCs and 2.5×10^6 epithelial cells combined with various concentrations of PRP into the chamber, we found that PRP (10% or 15%) shortened the time from dissociated
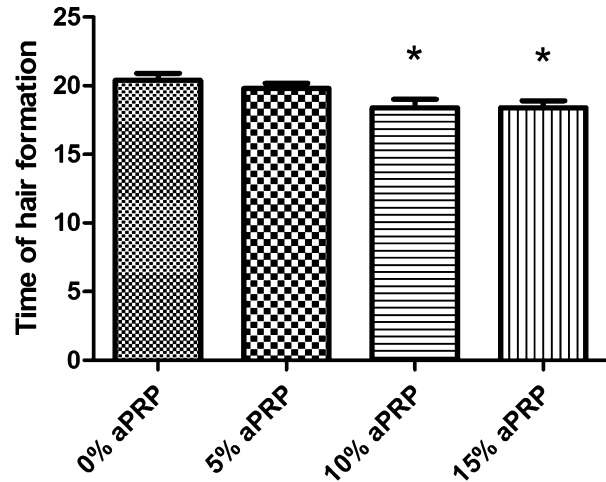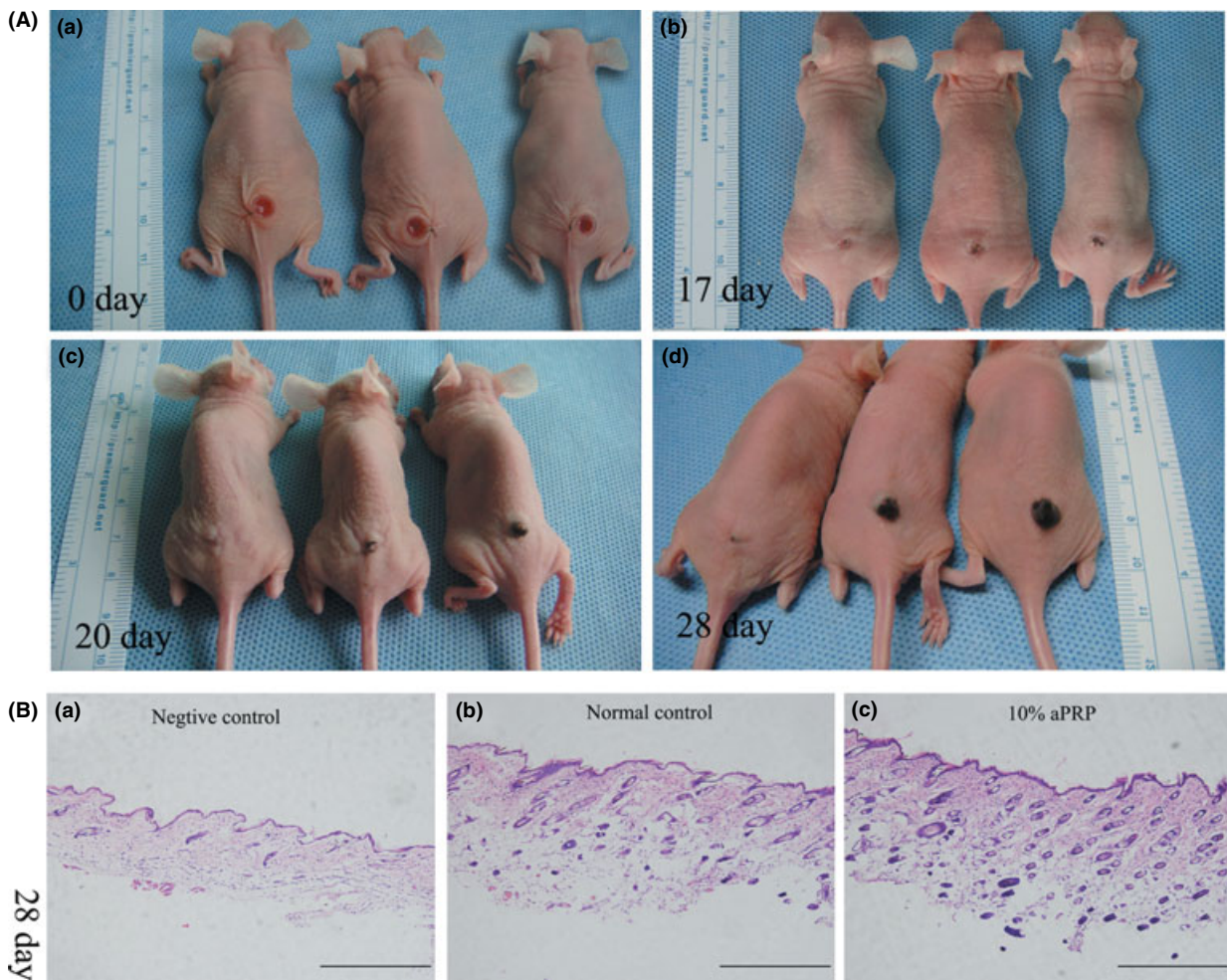
Figure 6. Effect of platelet-rich plasma (PRP) on hair follicle (HF) reconstitution of nude mice in the chamber assay. (A) Macroscopic appearance of reconstituted skin at various days after grafting. (a) Transplantation of cell suspension to the chamber of dorsal skins of nude mice in 0 days. (b) Seventeen days after grafting, the first hairs were observed on the grafted area in activated PRP (right animals in a, b, c, and d), whereas the negative control (left animals in a, b, c, and d) and the normal control (middle animals in a, b, c, and d) revealed melanization of only the epidermis. (c) Twenty days after grafting, hairs were observed on the normal control, whereas the negative control showed melanization of only the epidermis. (d) Twenty-eight days after grafting, abundant hairs were observed on normal control and activated PRP (aPRP) mice, but the latter had the appearance of thick hair, and the negative control showed melanization of only the epidermis. (B) Horizontal histologic section of the reconstituted skin 28 days after grafting. (a) Few or no HFs formed when the negative control was grafted. (b and c) Abundant HFs formed on the normal control and aPRP, but aPRP resulted in dramatically more HFs reconstituted than in the normal control (hematoxylin and eosin staining; magnification ×100). The bar represents 500 μm. EC, negative control; EC + DPCs, normal control; EC + DPCs + 10% PRP, PRP; ECs, epidermal cells.

for this rate difference is not clear, it might be that, in contrast to HF embryogenesis, new follicles do not form first in the chamber assay. Therefore, PRP can accelerate HF reconstitution because PRP contains various growth factors and has the ability to stimulate wound healing, which is similar to the results of earlier studies in which PRP was used in hair transplantation to promote healing.[12,20–24] Although PRP could shorten the time of hair formation from dissociated cells in a chamber assay, the concentration of PRP should reach 10% or 15%, not 5%.

The density of hair formed with 0% (288 ± 35) or 5% (297 ± 41) PRP was significantly lower than that formed with 10% (344 ± 27) or 15% (342 ± 19) PRP. Although the reason for this
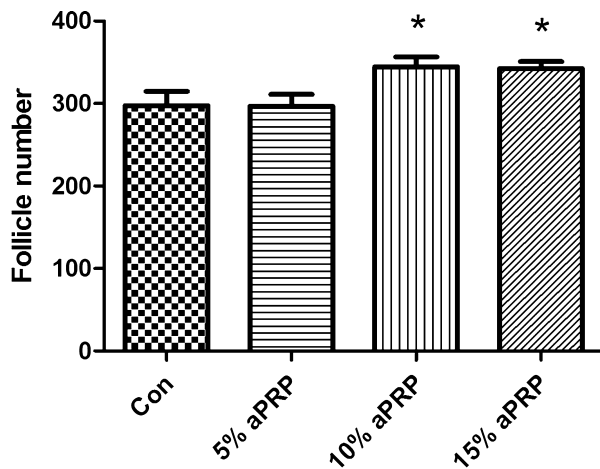
Figure 7. Effect of platelet-rich plasma (PRP) on the number of hair follicles (HFs) formed. The bar diagram shows the number of HFs formed from dissociated cells. The number of dermal papilla cells (DPCs) was fixed at 5×10^6 in a ratio of DPCs to epidermal cells of 2:1 for each grafting. The error bars represent standard deviations from five samples. *p < .05 vs the normal control. aPRP, activated PRP.

density difference is not clear, the explanation might be that blood vessels supply HFs with nutrients in vivo but that HFs also receive signals from autologous cells (autocrine effects) or neighboring cells (paracrine effects) through signaling molecules, such as growth factors. Loss or reduction of nutrients and signaling factors does not allow full development of HFs.[4,5] In the early phases of folliculoneogenesis (before angiogenesis), grafted cells can obtain only limited nutrition and growth factors from surrounding tissue fluid. Earlier studies have showed that there is extensive apoptotic remodeling in the early phases of grafting and that fewer cells contribute to the generation of a given follicle.[25] Thus, reducing apoptosis and improving the efficiency of grafted cells is an option in the early phase. For grafted cells, PRP contains various growth factors that supply sufficient signaling molecules (PDGD, FGF, and TGF-β) to promote cell proliferation and stimulate vascularization (VEGF, PDGF, EGF, TGF-β, and FGF), supplying sufficient nutrition for folliculoneogenesis.[6–12]

Similar to earlier studies showing that the use of PRP in hair transplantation leads to greater follicular density,[20–24] our study showed that the use of PRP in HF reconstitution leads to greater hair formation, but they might have different mechanisms; PRP can "wake up" dormant HFs in the scalp for the former, whereas PRP can improve the efficiency of follicular formation for the latter.

Hair follicle reconstruction requires a large number of DPCs, but DPCs will lose their hair-inductive capacity in culture with passage.[26] Therefore, to regenerate sufficient numbers of HFs, in addition to increasing the number of DPCs, we can improve the utilization rate of individual cells to improve the efficiency of HF reconstruction. According to the results obtained in this study, there was a 19.4% greater yield of HFs in the area of reconstituted skin treated using 10% PRP than with 0% PRP. This demonstrates that the use of PRP can improve the efficiency of HF reconstruction. Together with earlier reports,[12,13,20–24] our study demonstrates that PRP can promote hair formation. Thus, to improve the efficiency of HF reconstruction, we can try cotransplantation of DPCs and epidermal cells with PRP in a future study. PRP contains many growth factors (including PDGF, TGF-β-1/2, VEGF, IGF-1, EGF, and FGF)[6] that, in contact with their respective receptors, act in tissue angiogenesis, stimulating the healing and growth of new organic structures.[6–11] Some growth factors (TGF-β-1 and EGF) have negative effects on hair growth and formation,[4,5] whereas other growth factors, such as PDGF and VEGF, are important for hair formation and follicle size.[27,28] Numerous studies have demonstrated that the use of PRP in hair transplantation leads to accelerated hair growth and larger follicular units.[12,13,20–24] Thus, in accord with the results of earlier studies, the overall effect of all growth factors on hair is positive.

There are many different PRP systems, and the effects of these preparations can even be counterproductive.[29,30] Although the reason for this is not clear, it can depend on whether leucocytes are included in PRP preparations.[31] Apart from producing large amounts of VEGF,[32] leucocytes can release many

proinflammatory cytokines, which might have negative effects on hair growth. Leucocytes were included in the PRP in this study to harvest more platelets. To avoid the influence of leucocytes on hair growth, we used the supernatant from the centrifugation of activated PRP. Thus, although leucocytes were included in the PRP, they were not present in the final supernatant because they were pelleted in the third centrifugation. In addition, although stimulated leucocytes can release many cytokines, no leucocyte degranulation or damage occurred during sample preparation,[33–36] and after centrifugation of the activated PRP, the supernatant did not contain cytokines released from leucocytes. In the process of PRP preparation, to increase the purity of the PRP, we recommend increased thickness of PRP layer using centrifugal tubes with a smaller inner diameter and reduced flow rate of PRP using pipette tips with a larger inner diameter.

Conclusion

Using the chamber assay, this study observed a considerable effect of PRP on the time of hair formation and the yield of HF reconstitution. Considering the limited evidence of its efficacy, further studies are required to investigate the mechanism of action of PRP.

References

1. Rhodes T, Girman CJ, Savin RC, Kaufman KD, et al. Prevalence of male pattern hair loss in 18–49 year old men. Dermatol Surg 1998;24:1330–2.

2. Stenn KS, Cotsarelis G. Bioengineering the hair follicle: fringe benefits of stem cell technology. Curr Opin Biotechnol 2005;16:493–7.

3. Miteva M, Tosti A. Treatment options for alopecia: an update, looking to the future. Expert Opin Pharmacother 2012;13:1271–81.

4. Botchkarev VA, Paus R. Molecular biology of hair morphogenesis: development and cycling. J Exp Zool B Mol Dev Evol 2003;298:164–80.

5. Paus R, Cotsarelis G. The biology of hair follicles. N Engl J Med 1999;341:491–7.

6. Anitua E, Andia I, Ardanza B, Nurden P, et al. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost 2004;91:4–15.

7. Nikolidakis D, Jansen JA. The biology of platelet-rich plasma and its application in oral surgery: literature review. Tissue Eng Part B Rev 2008;14:249–58.

8. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. Plast Reconstr Surg 2004;114:1502–8.

9. de Vos RJ, van Veldhoven PL, Moen MH, Weir A, et al. Autologous growth factor injections in chronic tendinopathy: a systematic review. Br Med Bull 2010;95:63–77.

10. Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: a review of biology and applications in plastic surgery. Plast Reconstr Surg 2006;118:147e–59e.

11. Kim DH, Je YJ, Kim CD, Lee YH, et al. Can platelet-rich plasma be used for skin rejuvenation? Evaluation of effects of platelet-rich plasma on human dermal fibroblast. Ann Dermatol 2011;23:424–31.

12. Uebel CO, da Silva JB, Cantarelli D, Martins P. The role of platelet plasma growth factors in male pattern baldness surgery. Plast Reconstr Surg 2006;118:1458–66.

13. Li ZJ, Choi HI, Choi DK, Sohn KC, et al. Autologous platelet-rich plasma: a potential therapeutic tool for promoting hair growth. Dermatol Surg 2012;38:1–7.

14. Williams D, Profeta K, Stenn KS. Isolation and culture of follicular papillae from murine vibrissae: an introductory approach. Br J Dermatol 1994;130:290–7.

15. Lichti U, Anders J, Yuspa SH. Isolation and short-term culture of primary keratinocytes, hair follicle populations and dermal cells from newborn mice and keratinocytes from adult mice for in vitro analysis and for grafting to immunodeficient mice. Nat Protoc 2008;3:799–810.

16. Weinberg WC, Goodman LV, George C, Morgan DL, et al. Reconstitution of hair follicle development in vivo: determination of follicle formation, hair growth, and hair quality by dermal cells. J Invest Dermatol 1993;100:229–39.

17. Liang Y, Silva KA, Kennedy V, Sundberg JP. Comparisons of mouse models for hair follicle reconstitution. Exp Dermatol 2011;10:1–5.

18. Jensen KB, Driskell RR, Watt FM. Assaying proliferation and differentiation capacity of stem cells using disaggregated adult mouse epidermis. Nat Protoc 2010;5:898–911.

19. Driskell RR, Giangreco A, Jensen K, Mulder KW, et al. Sox2-positive dermal papilla cells specify hair follicle type in mammalian epidermis. Development 2009;136:2815–23.

20. Perez-Meza D. Part II The use of autologous rich and poor plasma to enhance the wound healing and hair growth in hair restoration. In programs and Abstracts. 13th ISHRS annual meeting; Sydney, Australia 2005.

21. Uebel C. A new advance in baldness surgery using platelet-derived growth factor. Hair Transplant Forum Int 2005;15:77–84.

22. Perez-Meza D, Leavitt M, Mayer M. The growth factors Part 1: clinical and histological evaluation of the wound healing and

revascularization of the hair graft after hair transplant surgery. Hair Transplant Forum Int 2007;17:173–5.

23. Greco J, Brandt R. Preliminary experience and extended applications for the use of autologous platelet rich plasma in hair transplantation surgery. Hair Transplant Forum Int 2007;17:131–2.

24. Greco J, Brandt R. The effects of autologous platelet rich plasma and various growth factors on non-transplanted miniaturized hair. Hair Transplant Forum Int 2009;19:49–50.

25. Zheng Y, Du XB, Wang W, Boucher B. Organogenesis from dissociated cells: generation of mature cycling hair follicles from skin-derived cells. J Invest Dermatol 2005;124:867–76.

26. Jahoda CA, Horne KA, Oliver RF. Induction of hair growth by implantation of cultured dermal papilla cells. Nature 1984;311:560–2.

27. Takakura N, Yoshida H, Kunisada T, Nishikawa S, et al. Involvement of platelet derived growth factor receptor-a in hair canal formation. J Invest Dermatol 1996;107:770–7.

28. Yano K, Brown L, Detmar M. Control of hair growth and follicle size by VEGF-mediated angiogenesis. J Clin Invest 2001;107:409–17.

29. Graziani F, Ivanovski S, Cei S, Ducci F, et al. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. Clin Oral Implants Res 2006;17:212–19.

30. Cenni E, Ciapetti G, Pagani S, Perut F, et al. Effects of activated platelet concentrates on human primary cultures of fibroblasts and osteoblasts. J Periodontol 2005;76:323–8.

31. Dohan ED, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009;27:158–67.

32. Werther K, Christensen IJ, Nielsen HJ. Determination of vascular endothelial growth factor (VEGF) in circulating blood: significance of VEGF in various leucocytes and platelets. Scand J Clin Lab Invest 2002;62:343–50.

33. Everts PA, Hoffmann J, Weibrich G, Mahoney CB, et al. Differences in platelet growth factor release and leucocyte kinetics during autologous platelet gel formation. Transfus Med 2006;16:363–8.

34. Everts PA, Jakimowicz JJ, van Beek M, Schönberger JP, et al. Reviewing the structural features of autologous platelet-leukocyte gel and suggestions for use in surgery. Eur Surg Res 2007;39:199–207.

35. Moojen DJ, Everts PA, Schure RM, Overdevest EP, et al. Antimicrobial activity of platelet-leukocyte gel against Staphylococcus aureus. J Orthop Res 2008;26:404–10.

36. Dohan Ehrenfest DM, Corso MD, Diss A, Mouhyi J, et al. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol 2010;81:546–55.

Address correspondence and reprint requests to: Zhi-Qi Hu, Department of Plastic and Aesthetic Surgery, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China, or e-mail: doctorhzq@hotmail.com