Platelets, November 2010; 21(7): 549–554

informa
healthcare

ORIGINAL ARTICLE

Platelet gel from cord blood: A novel tool for tissue engineering

VALENTINA PARAZZI, LORENZA LAZZARI, & PAOLO REBULLA

Cell Factory, Center of Transfusion Medicine, Cellular Therapy and Cryobiology, Department of Regenerative Medicine, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

(Received 7 July 2010; revised 5 August 2010; accepted 6 August 2010)

Abstract
Recent findings show that growth factors (GF) play a relevant role in regenerative medicine. Platelets (PLT) may be used as ''drug-stores'' of GF that can be released upon activation by PLT granules. In this context, PLT gel (PG) from peripheral blood is currently used to improve tissue healing in orthopedic, oral maxillofacial and dermatologic surgery. Recent findings on multiple biological properties of human umbilical cord blood (CB) and its high level of viral safety prompted us to investigate the characteristics of its PLTs and the possibility to produce PLT gel from cord blood. Our study shows that CB PG releases high levels of vascular endothelial growth factor (VEGF) and platelet-derived growth factor-BB (PDGF-BB), substantial amounts of fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and transforming growth factor-beta 1 (TGFbeta1), and minimal amounts of PDGF-AB. These findings suggest that CB PG can be a preferable tool for tissue engineering applications where high levels of VEGF and PDGF may be desirable.

Keywords: *Platelet gel, growth factors, cord blood, peripheral blood*

Introduction

Umbilical cord blood (CB) is an important alternative source of hematopoietic stem cells for allogenic transplantation in children and adults with cancer, bone marrow failure syndromes, hemoglobinopathies and many genetic metabolic disorders [1]. In the last 20 years several cord blood banks have been created worldwide in order to collect and store related and unrelated CB units for the treatment of hematological disorders [2–4]. Today, there are more than 100 active CB banks with an inventory of more than 400,000 CB units that have generated more than 10,000 CB transplants all around the world [5]. Moreover, it has recently been shown that CB is also a rich source of human mesenchymal stem cells (MSC), which seem to have tissue regenerative and immunosuppressive properties that could make them an important candidate for cell therapy approaches [6]. Finally, CB is toxoplasma, cytomegalovirus free and less immunogenic than other sources such as bone marrow and fat.

In spite of the consolidated use of CB in allogeneic hematopoietic transplant, knowledge is limited on the therapeutic potential of CB platelets (PLTs). Conversely, PLTs from peripheral blood have been deeply studied for their role in hemostasis [7] and more recently for their clinical use for wound healing in diabetic patients [8] and in bone repair [9].

These effects are largely due to growth factors (GF) contained in the platelet granules – platelet-derived growth factor (PDGF), transforming growth factor (TGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) – which have been shown to contribute to tissue regeneration [10, 11].

GF release takes place during platelet activation, which can be triggered by calcium gluconate and human thrombin or – more recently – by batroxobin derived from *Bothrops atrox* snake venom [12]. Similarly to thrombin, batroxobin promotes both PLT degranulation and the coagulation cascade. The process results in the formation of a gelatin like product, called platelet gel (PG), in which PLTs are

Correspondence: Paolo Rebulla, MD, ''Franco Calori'' Cell Factory, Center of Transfusion Medicine, Cellular Therapy and Cryobiology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122 Milano, Italy. Tel: +39 02 5503 4021. Fax: +39 02 5458129. E-mail: prebulla@policlinico.mi.it

ISSN 0953–7104 print/ISSN 1369–1635 online
DOI: 10.3109/09537104.2010.514626

trapped within the fibrin network where they continue to secrete their contents and bioactive substances. The high safety profile of commercial batroxobin has been shown in a number of clinical trials in which batroxobin has been used for its fibrinogenic effect [13–17].

Although PLT releasate in the form of activated PG derived from adult peripheral blood has been extensively used for topical therapy of various clinical conditions, including wounds and soft-tissue injuries, we are not aware of experimental observations on PG from CB.

The aim of this study was to prepare, standardize and characterize CB PG with a focus on the evaluation of GF released by this new blood component.

Methods

Sample collection

CB units were collected after parental informed consent in plastic bags containing 29 mL of citrate-phosphate-dextrose (CPD) anticoagulant by trained midwives, before and after placenta delivery in term natural deliveries and cesarean sections, respectively, according to a procedure described in detail elsewhere [18]. After storage at room temperature (RT), the units were processed within 24 ± 2 hours of collection.

Platelet gel preparation for in vitro study

CB was transferred into centrifuge tubes, centrifuged at $550 \times g$ for 10 min at RT and the top platelet-rich plasma fraction (PRP) was collected. A complete blood count was made on the PRP using an automated hematology analyzer.

If nucleated cell (NC) count exceeded 1×10^5/mL, the sample was centrifuged again at $100 \times g$ at RT for 10 min. The supernatant PRP was collected and the pellet containing most nucleated cells was discarded.

Then, the PRP was centrifuged at $2000 \times g$ for 15 min at RT and the PLT pellet was resuspended in an adequate volume of supernatant platelet poor plasma (PPP) to obtain a final concentration of 2×10^6 PLTs/μL. Then, the PRP was left to stand for 30 min at RT and finally activated using calcium gluconate and batroxobin (Plateltex-Act, Plateltex, Bratislava, Slovakia). The Plateltex gel kit included a vial of liophylized batroxobin that must be dissolved in 1 mL of calcium gluconate (Monico, Italy) immediately before use (PRP/activator ratio 9:1) [19]. Consolidation of the PG occurred in about 10 minutes.

The PG was centrifuged at $2000 \times g$ for 15 min at RT to obtain a supernatant rich of growth factors released by the activated PLTs. The PLT releasate was filtered using a 0.22 μm cell strainer to remove PLT debris and the amount of released GF was measured. PPP was used as a growth factor-poor control (blank, see below).

Growth factor detection

Commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, Minneapolis, MN, USA) were used according to the manufacturer's instructions to quantify the concentrations of platelet-derived growth factor–AB (PDGF-AB), transforming growth factor-beta 1 (TGFbeta1) and vascular endothelial growth factor (VEGF). Six PLT releasates and corresponding PPPs were collected from six individual CB unit PG preparations and stored at –20°C until evaluation.

Each well of the microplate was pre-coated with target protein-specific antibodies. The standard and sample solutions were added to the plate. After the corresponding incubation time, the plate was washed with a washing buffer and a solution of corresponding polyclonal antibodies conjugated to horseradish peroxidase was added. After incubation and washing, the substrate solution was added to start the enzyme-catalyzed reaction, which was stopped by the addition of a stop solution.

The optical density of each sample was determined using a microplate reader at 450 nm (Genios Plus, TECAN, Salzburg, Austria). The PPP was also tested as a blank and the value obtained was subtracted form the amount of factor present in PLT releasate. Standard dilutions of isolated GF were used to create calibration curves.

Data were analysed using Magellan Software and expressed as mean picograms of the secreted factors per mL at the time of harvest.

Other factors were detected by a multiplexed sandwich ELISA that allowed quantitative chemiluminescent measurement of four proteins per well (PDGF-BB, VEGF, bFGF, hepatocyte growth factor (HGF); their lower detection limits were 1.0; 4.9; 2.0; 3.1 pg/mL, respectively) (SearchLight proteome array, Aushon Biosystems, Billerica, MA, USA).

Each well of the microplate was pre-spotted with target protein-specific antibodies and 50 μL of standards and samples were added to the plate for 1 hour at RT with shaking at 200 rpm. After well washing to remove unbound proteins, 50 μL of biotinylated antibodies were added to the plate for 30 min at RT with shaking at 200 rpm. Excess biotynilated antibody was washed away, and 50 μL of streptavidin-horseradish peroxidase (SA-HRP) was added to the plate for 30 min at RT with shaking at 200 rpm.

In order to identify the signal, we used SuperSignal ELISA femto chemiluminescent substrate (Pierce, Rockford, IL, USA). The luminescent signal was recorded within 10 min using a cooled

CCD camera and the amount of each target protein was analysed using Microsoft Excel 2000 as recommended by the manufacturers. The PPP was also tested as a blank and the value obtained was subtracted from the amount of factor present in the PLT releasate.

Platelet gel preparation for clinical applications

To facilitate CB PG storage and transportation in aseptic conditions, we developed a simple procedure in a closed system for clinical applications. CB units of the same AB0 group were pooled in a 500 mL transfer bag (JMS Corporation, Friedrichsdorf, Germany) to achieve a volume of 200–250 mL, and centrifuged at 840*g* for 4 minutes (RT). The PRP was transferred into a new bag and a blood count was made to determine the PLT count. The PRP was centrifuged at 3122*g* for 15 min and the PLT pellet was resuspended in an adequate volume of PPP to obtain a final concentration of 2×10^6 PLTs/μL.

Subsequently, PRP was left overnight at RT with gentle agitation. The following day, the PRP was split in 50 mL biobags and stored at −80°C for some weeks before thawing and activation (Fresenius Kabi AG, Hamburg, Germany). Activation was performed using calcium gluconate and batroxobin (Plateltex-Act) as reported above.

Statistical analysis

The data are presented as mean and standard deviation (SD).

Results

Cord blood platelet gel production

A total of 40 CB PG were prepared. The PLT count in CB units at collection was $202 \pm 43 \times 10^3$ per μL (mean ± SD).

Mixing PRP with batroxobin and calcium gluconate solution to antagonize the anticoagulant effect of citrate present in CB bags resulted in the activation of the PLT concentrate and the development of a viscous, thick and adhesive PG film (Figure 1A).

The system of PG preparation for in vitro studies was easily reproducible in all the tested samples. Similarly, CB PG production in a closed system in view of clinical applications was successful in each preparation (Figure 1B). From about 250 mL of cord blood (same ABO group), 16–20 mL of CB PG were obtained. Monitoring clot retraction after batroxobin activation showed minimal retraction after 40 minutes and this did not increase within the next 24 hours.

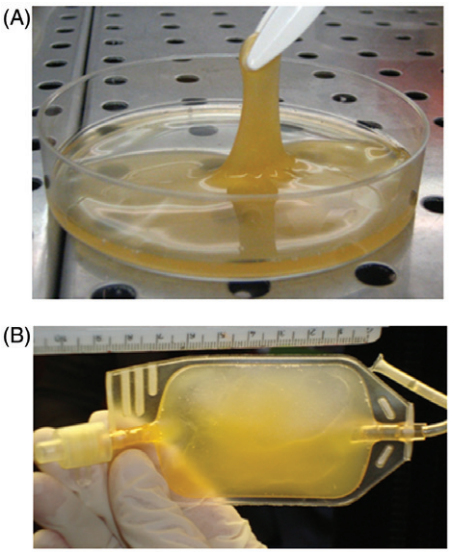

Figure 1. (A) Cord blood platelet gel for in vitro study. (B) Cord blood platelet gel for clinical applications.

Table I. PDGF-AB and TGFbeta1 concentrations in PPP and CB PG releasate. Growth factor concentration measured by ELISA assay (pg/mL; $n = 6$; mean ± SD)

	Growth factors	
	PDGF-AB	TGF beta 1
PPP	14.2 ± 2.4	830.6 ± 222.5
*PG releasate**	41 ± 23.3	6865.6 ± 2668.5

*Value obtained by subtracting PPP from total CB PG releasate.

Table II. PDGF-BB, HGF, bFGF and VEGF concentrations in PPP and CB PG releasate. Growth factor concentration measured by SearchLight assay (pg/mL; $n = 6$; mean ± SD).

	Growth factors			
	PDGF-BB	HGF	FGFb	VEGF
PPP	323.6 ± 23	294 ± 20	2.1 ± 0.4	363.6 ± 42
*PG releasate**	11083.9 ± 5443.8	610.7 ± 254	85.5 ± 21.4	1803.5 ± 788.5

*Value obtained by subtracting PPP from total CB PG releasate.

Evaluation of growth factors

The amount of growth factors was measured in six individual PLT releasates of CB PGs. In particular, concentrations of TGFbeta1 and PDGF-AB, which were evaluated by ELISA in the CB PG releasate, are reported in Table I. The levels of PDGF-BB, VEGF, bFGF and HGF, which were determined with a chemiluminescent quantitative measurement, are reported in Table II.

Discussion

PLTs are non-nucleated cellular fragments that release the contents of their intracellular granules in response to activation. PLT secretory granules contain growth factors, coagulation proteins, cell-activating molecules and some other molecules, which are synthesized in megakaryocytes and packaged into the granules through vescicle-trafficking processes [20]. Peripheral blood PLTs are a well known starting material to obtain PG, a blood component that can be used in several regenerative treatments including chronic wound, skin and soft tissue repair [21]. More recently, PG has been considered also for breast cosmetic augmentation and in anti-aging medicine [22]. Several methods for PG preparation have been reported in the literature, some based on commercially available kits and some on local laboratory procedures. Important features of the different methods are the activator (calcium gluconate, autologous thrombin or batroxobin) and centrifugation strategy. After PLT activation, some of the numerous growth factors including PDGF, TGF, FGF, VEGF and HGF are released by the PLT granules and these factors are responsible for the chemotactic and mitogenic properties that promote and modulate cellular functions involved in soft and hard tissue healing and cell proliferation. Based on these PLT properties, clinical studies have reported improvements of wound healing after treatment with peripheral blood PG and PLT growth factors [8, 11].

In this study, our main interest was to determine if PG from CB – a readily available, virtually virus free platelet source – showed important differences in the concentration of growth factors known to play a stimulatory role in in vitro cell culture and in vivo tissue repair, as compared to PG from adult peripheral blood.

Based on recent observations [23] and with the goal to define a highly reproducible method independent from concentrations of autologous activators, we decided to produce CB PG through the addition of a standard reagent (Plateltex). Of note, this system has been already shown to release a significant amount of growth factors, in particular bFGF and EGF [24, 25].

Herein we demonstrated that a PG with a viscosity and thickness comparable with those obtained from adult peripheral blood PLTs was obtained from all processed CB units.

In view of regenerative medicine applications, the efficacy of PG is mediated by the release of growth factors that can act on tissue repair via paracrine effects. Currently, it is still quite difficult to compare the results produced by different groups regarding the secretion of PLT growth factors, since different methods have been used to induce their release. The most frequently used method combines steps of freezing at –80°C and thawing at 37°C of the PLT concentrate samples that are manually performed without any PLT activation. This process causes platelet destruction and the subsequent release of growth factors, but it is operator dependent and with high variability due to the manual procedure. The other method, used in this study, is based on batroxobin and calcium Gluconate, which are added to the GF concentrate to trigger GF release. The latter approach has less variability and is more standardizable than the previous one, thus it produces more reliable growth factor levels. Moreover, it is known that batroxobin-induced PRP activation releases PLT trophic factors more slowly than other methods, supporting their longer in loco activity [19]. Therefore, in view of future regenerative medicine applications this different kinetic of growth factors bioavailability could be appropriate to treat different kinds of lesions or different kinds of tissues when a prolonged release is necessary.

In addition, in order to provide valid results, we used an innovative multiplex sandwich technology and a classical ELISA assay. The first one has a higher sensitivity if compared with other standard colorimetric platforms and the second one is a validated method used for in vitro diagnostic applications. The multiplex sandwich technology was selected based on some recently published articles [26] and it was used mainly because multiplex arrays offer opportunities to examine physiologically relevant panel(s) of cytokines or entire ''classes'' of cytokines in a time and cost-efficient fashion.

Our results showed that CB PG is rich in growth factors. In particular, we found high levels of VEGF and PDGF-BB, substantial amounts of FGF, HGF and TGFbeta1, and only minimal amounts of PDGF-AB, as reported in Tables I and II.

Interestingly, we found a very high level of VEGF in CB PG releasate as compared with the results obtained using the same activator by Mazzucco et al. [23] (1803.5 ± 788.5 pg/mL vs 250 ± 120 pg/mL, respectively). In order to confirm this significant difference of the VEGF content between adult peripheral blood PG and CB PG, we also validated these results using the ELISA assay used by Mazzucco et al. (1645.3 ± 891.58 pg/mL in CB PG releasate). These different levels of VEGF between peripheral blood PG and CB PG could be explained by extrapolating the properties of CB stem cells as part of perivascular/endothelial compartment [27–29] to CB PLTs. Several clinical/regenerative applications could benefit from this high amount of VEGF released by CB–PG, including the treatments of diabetic complications characterized by impaired neovascularization. In fact, diminished production of VEGF and decreased angiogenesis are thought to contribute to impaired tissue repair in diabetic patients. VEGF-treated wounds demonstrated

increased epithelialization, increased matrix deposition and enhanced cellular proliferation [30, 31].

On the other side, we observed low levels of TGFbeta1 in CB PG releasate as compared with the peripheral blood counterpart where this growth factor is highly expressed (6865.6 ± 2668.5 pg/mL vs 11250 ± 6796). TGF-beta1 is a multifunctional regulatory protein capable of inducing site-specific healing responses by increasing collagen synthesis and deposition. It is a very potent stimulator of osteoblastic bone formation, causing chemotaxis, proliferation and differentiation in committed osteoblasts [32, 33].

Therefore, on the basis of our results, we suggest that CB PG could be used in an ABO compatible setting for the treatment of a large number of medical conditions including skin sores, necrotic vascular and a-vascular lesions. In addition, due to the high levels of angiogenic factors, CB PG could be more prone to support endothelial applications than peripheral blood PG in different clinical settings.

Conclusion

In conclusion, herein we demonstrate the feasibility to obtain PG from CB. We believe that CB PG may be an interesting and safe source of growth factors for clinical applications, in particular in oral, maxillofacial and periondontal surgery and in the treatment of skin ulcers.

Acknowledgment

This study was supported by grants from the Italian Ministry of Health (Progetto a Concorso 2008 and 2009, Ex Art. 56), Fondazione Il Sangue, the Istituto Superiore di Sanità (Malattie Neurodegenerative), the 6FP EU Project THERCORD, the 7FP EU Project CASCADE and the 7FP EU Project REBORNE.

References

1. Gluckman E. History of cord blood transplantation. Bone Marrow Transplant 2009;44:621–626.
2. McGuckin CP, Forraz N. Umbilical cord blood stem cells-an ethical source for regenerative medicine. Med Law 2008;27:147–165.
3. Brand A, Rebulla P, Engelfriet CP, Reesink HW, Beguin Y, Baudoux E, Kögler G, Ebrahimi M, Grazzini G, Costa AN, et al. Cord blood banking. Vox Sang 2008;95:335–348.
4. Rubinstein P. Cord blood banking for clinical transplantation. BMT 2009;44:635–642.
5. Garcia J. Allogeneic unrelated cord blood banking worldwide: An update. Transfus Apher Sci 2010;42:257–263.
6. Kode JA, Mukherjee S, Joglekar MV, Hardikar AA. Mesenchymal stem cells: Immunology and role in immunomodulation and tissue regeneration. Cytotherapy 2009;11:377–391.
7. Jurk K, Kehrel BE. Platelets: Physiology and biochemistry. Semin Thromb Hemost 2005;31:381–392.
8. Driver VR, Hanft J, Fylling CP, Beriou JM. A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. Ostomy Wound Manage 2006;52:68–74.
9. Gruber R, Varga F, Fischer MB, Watzek G. Platelets stimulate proliferation of bone cells: Involvement of platelet-derived growth factor, microparticles and membranes. Clin Oral Implants Res 2002;13:529–535.
10. 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;91:4–15.
11. Frechette JP, Martineau I, Gagnon G. Platelet-rich plasmas: growth factor content and roles in wound healing. J Dent Res 2005;84:434–439.
12. Carr ME, Carr SL, Tildon T, Fisher LM, Martin EJ. Batroxobin-induced clots exhibit delayed and reduced platelet contractile force in some patients with clotting factor deficiencies. J Thromb Haemost 2003;1:243–249.
13. Kubo T, Matsunaga T, Asai H, Kawamoto K, Kusakari J, Nomura Y, Oda M, Yanagita N, Niwa H, Uemura T, et al. Efficacy of defibrinogenation and steroid therapies on sudden deafness. Arch Otolaryngol Head Neck Surg 1988;114:649–652.
14. Gusev EI, Skvortsova VI, Suslina ZA, Avakian GN, Martynov Mlu, Temirbaeva SL, Tanashian MA, Kamchtnov PR, Stakhovskaia LV, Efremova NM. Batroxobin in patients with ischemic stroke in the carotid system (the multicenter study). Zh Nevrol Psikhiatr Im S S Korsakova 2006;106:31–34.
15. Wang HT, Jiang WL, Zhang YN, Sun ZF, Sun QF, Ma J. Multicentre clinical observation of anticoagulation and thrombolysis for the deep venous thrombosis. Zhonghua Yi Xue Za Zhi 2009;89:3181–3185.
16. Zeng Z, Xiao P, Chen J, Wei Y. Are batroxobin agents effective for perioperative hemorrhage in thoracic surgery? A systematic review of randomized controlled trials. Blood Coagul Fibrinolysis 2009;20:101–107.
17. Zhang L, Lu SH, Li L, Tao YG, Wan YL, Senga H, Yang R, Han ZC. Batroxobin mobilizes circulating endothelial progenitor cells in patients with deep vein thrombosis. Clin Appl Thromb Hemost 2009; Oct 13. [Epub ahead of print].
18. Sirchia G, Rebulla P, Mozzi F, Lecchi L, Lazzari L, Ratti I. A quality system for placental blood banking. Bone Marrow Transplant 1998;21:843.
19. Mazzucco L, Balbo V, Cattana E, Borzini P. Platelet-rich plasma and platelet gel preparation using Plateltex®. Vox Sang 2008;94:202–208.
20. Garcia A, Zitzmann N, Watson SP. Analyzing the platelet proteome. Semin Thromb Hemost 2004;30:485–489.
21. Everts PA, Knape JT, Weibrich G, Schonberger JP, Hoffmann J, Overdevest EP, Box HA, van Zundert A. Platelet-rich plasma and platelet gel: A review. J Extra Corpor Technol 2006;38:174–187.
22. Man D, Plosker H, Winland-Brown JE. The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. Plast Reconstr Surg 2001;107:229–237.
23. Mazzucco L, Balbo V, Cattana E, Guaschino R, Borzini P. Not every PRP-gel is born equal. Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet, RegenPRP-Kit, Plateltex and one manual procedure. Vox Sang 2009;97:110–118.
24. Kurane A, Vyavahare N. In vivo vascular tissue engineering: Influence of cytokine and implant location on tissue specific cellular recruitment. J Tissue Eng Regen Med 2009;3:280–289.
25. Tamama K, Kawasaki H, Wells A. Epidermal growth factor (EGF) treatment on multipotential stromal cells (MSCs). Possible enhancement of therapeutic potential of MSC. J Biomed Biotechnol Volume 2010 (2010), Article ID 795385.

26. Backen AC, Cummings J, Mitchell C, Jayson G, Ward TH, Dive C. 2009. 'Fit -for-purpose' validation of SearchLight multiplex ELISAs of angiogenesis for clinical trial use. J Immunol Methods 2009;342:106–114.
27. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3:301–313.
28. Chen CW, Montelatici E, Crisan M, Corselli M, Huard J, Lazzari L, Péault B. Perivascular multi-lineage progenitor cells in human organs: Regenerative units, cytokine sources or both? Cytokine Growth Factor Rev 2009;20:429–434.
29. Morigi M, Rota C, Montemurro T, Montelatici E, Lo Cicero V, Imberti B, Abbate M, Zoja C, Cassis P, Longaretti L, et al. Life-sparing effect of human cord blood-mesenchymal stem cells in experimental acute kidney injury. Stem Cells 2010;28:513–522.
30. Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, Bunting S, Steinmetz HG, Gurtner GC. Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. Am J Pathol 2004;164:1935–1947.
31. Bao P, Kodra A, Tomic-Canic M, Golinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. J Surg Res 2009;153:347–358.
32. Beck LS, Deguzman L, Lee WP, Xu Y, McFatridge LA, Gillett NA, Amento EP. Rapid publication. TGF-beta1 induces bone closure of skull defects. J Bone Miner Res 1991;6:1257–1265.
33. Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, Zhao L, Nagy TR, Peng X, Hu J, et al. TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. Nat Med 2009;15:757–765.