Journal of Cranio-Maxillo-Facial Surgery 47 (2019) 668–675

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

An *in vitro* long-term study of cryopreserved umbilical cord blood-derived platelet-rich plasma containing growth factors—PDGF-BB, TGF-β, and VEGF

Kyoko Baba [a, b, *], Yasuharu Yamazaki [a], Yumiko Sone [a], Yoshika Sugimoto [a], Kazuno Moriyama [a], Takayuki Sugimoto [a], Kennichi Kumazawa [a], Yasuhito Shimakura [a], Akira Takeda [a]

[a] Department of Plastic and Aesthetic Surgery (Head of Department: Prof. Dr. A. Takeda), School of Medicine, Kitasato University, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan
[b] Department of Plastic Surgery (Head of Department: Dr. K. Baba), Kitasato University Medical Center, 6-100 Arai, Kitamoto, Saitama 364-8501, Japan

ARTICLE INFO

Article history:
Paper received 1 August 2018
Accepted 15 January 2019
Available online 22 January 2019

Keywords:
Umbilical cord blood
Platelet-rich plasma
Growth factor
Mesenchymal stromal cells
Cryopreservation
Regenerative medicine

ABSTRACT

Purpose: Umbilical cord blood-derived platelet-rich plasma (UCB-PRP) containing growth factors has attracted attention as a biomaterial useful for regenerative medicine. The osteoblastic differentiation of umbilical cord-derived mesenchymal stromal cells (UC-MSCs) can be induced by UCB-PRP.
Materials and methods: Nine samples of UC and UCB were used to conduct an *in vitro* study that determined the contents of three growth factors (i.e., platelet-derived growth factor, transforming growth factor β-1, and vascular endothelial growth factor) and that examined, by staining with Alizarin red, their ability to induce the osteoblastic differentiation of UC-MSCs at the baseline, 3 months, and 3 years of cryopreservation.
Results: The contents of growth factors in cryopreserved UCB-PRP were markedly elevated compared to those found in UCB at baseline. The samples of UCB that were added with cryopreserved UCB-PRP and those with bone morphogenetic protein-2 were stained granularly with Alizarin red, thus indicating the presence of calcium. The samples of UCB that were not added with UCB-PRP were not stained with Alizarin red. The above-mentioned contents and ability were maintained at 3 years of cryopreservation. Cryopreserved UCB-PRP possibly and advantageously induced the osteoblastic differentiation of UC-MSCs.
Conclusion: The potential clinical application of cryopreserved UCB-PRP to regenerative medicine was suggested.

1. Introduction

Cells, scaffolds, and growth factors have conventionally been considered as the “three elements” of regenerative medicine (Yamada et al., 2011). Attention has been paid to growth factors because of their critical role in cytogenesis and histogenesis. Bone morphogenetic protein (BMP), one growth factor, is generally recognized to accelerate osteogenesis. However, BMP involves challenges to be addressed for its clinical application because of its adverse reactions (Coffman et al., 2016; Pereira et al., 2014; Tannoury et al., 2014; Zara et al., 2011) and its biological limitations when used alone (McLean et al., 2011; van den Dolder et al., 2006). In recent years, platelet-rich plasma (PRP) containing growth factors has attracted attention as a biomaterial useful for regenerative medicine. PRP, which is a physiological biomaterial (Dohan et al., 2018) and contains various types of platelet-derived growth factors, can be expected to exert the actions of multiple growth factors that are required for histogenesis (Mendonça-Caridad et al., 2006; Mooren et al., 2010) without the artificial enhancement of a single growth factor (Murphy et al., 2012). Furthermore, PRP is devoid of any issues related to cell

* Corresponding author. Department of Plastic and Aesthetic Surgery, School of Medicine, Kitasato University, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan. Fax: +81 42 788 9327.
E-mail address: kyokobaba@med.kitasato-u.ac.jp (K. Baba).

https://doi.org/10.1016/j.jcms.2019.01.020

transplantation because of not containing cells, and is easily preparable by simple procedures. Therefore, autologous peripheral blood-derived PRP (PB-PRP) is already in clinical use (Hsu et al., 2013; Kassolis et al., 2000; Marx, 2004; Marx et al., 1998; Mishra et al., 2012; Rodriguez et al., 2003; Murphy et al., 2017).

The authors are investigating the osteoblastic differentiation potential of mesenchymal stromal cells (MSCs) for the objective of improving osteogenesis in bone defects at the site of alveolar clefts of patients with cleft lip and palate. When intended to apply MSCs clinically, the authors consider that autologous tissues are preferable from the viewpoints of safety and ethics. The umbilical cord (UC) and umbilical cord blood (UCB) are utilizable as autologous tissues and can be collected noninvasively and easily at the time of birth. Furthermore, a large volume of UCB can be obtained at the time of birth, in contrast to peripheral blood, which cannot be obtained in large quantity from neonates, infants, and young children. Furthermore, PRP derived from adult peripheral blood is effective for the proliferation and calcification of MSCs derived from adult tissues (Murphy MS et al., 2017). These facts drove us to explore the possibility that UCB-derived PRP (UCB-PRP) can be utilized as a supply source of growth factors for bone regeneration.

Growth factors contained in UCB are potentially different from those contained in adult peripheral blood (van den Dolder et al., 2006; Murphy, 2012). UCB-PRP, which is collected at the time of birth, needs to be cryopreserved until the time of use. To the extent of the search, some studies on growth factors were found that are contained in UCB-PRP (Murphy et al., 2012; Rebulla et al., 2016; van den Dolder et al., 2006) but we have not found any study on UCB-PRP cryopreserved for a long-period of time. Against the above-mentioned background of research, the authors determined the contents of three growth factors in UCB-PRP that had been cryopreserved for 3 years or longer: 1) platelet-derived growth factor-BB (PDGF-BB)—an MSC proliferation accelerator; 2) transforming growth factor β1 (TGF-β1) of TGF-β superfamily that is similar in structure to BMP—an extracellular matrix production accelerator; and 3) vascular endothelial growth factor (VEGF)—an angiogenesis accelerator. Furthermore, the authors used UC-derived MSCs (UC-MSCs) that had been obtained from the same neonates as those who supplied UCB-PRP and had been cryopreserved for 3 years or longer in an attempt to examine the ability of growth factors to induce the osteoblastic differentiation of UC-MSCs *in vitro*. In the present study, which is preliminary and unique, the authors determined the contents of growth factors in UCB-PRP cryopreserved for a long period of time and examined their ability to induce the osteoblastic differentiation of UC-MSCs *in vitro*.

2. Materials and methods

The present study, approved by the ethics committee at Kitasato University (approval number B-07-13), was conducted after the acquisition of written informed consent from pregnant women. Tissues, namely UC and UCB, which are usually discarded in clinical practice, were collected as study materials under the conditions in which the safety of neonates and parturient women was ensured after the expulsion of the placenta. The collected samples of UC and UCB were numbered in order not to allow individual identification.

2.1. Treatment of UCB

The UC and UCB samples were collected from neonates who were born at weeks 37–41 of gestation without any problems during delivery, to mothers whom obstetricians had not found any maternal or fetal complications during pregnancy. Samples other than these were excluded. UCB was treated as per a previously reported method (Baba et al., 2013) (Fig. 1). Briefly, UCB was collected by puncturing the umbilical vein after the expulsion of the placenta. Immediately after collection, UCB was mixed with ACD-A (Terumo Co., Ltd., Tokyo, Japan) for anticoagulation. Subsequently, the mixture was centrifuged twice (2,400 rpm, 10 min; 3,600 rpm, 10 min) at 20 °C to obtain UCB-PRP and UCB-derived platelet-poor plasma (Fig. 2). UCB-PRP was cryopreserved at −80 °C until the time of use. UCB-PRP, thawed at room temperature just before use, was randomly allocated to the experiments (Table 1).

2.2. Concentration rates of UCB and the quantitative determination of growth factors

Immediately after collection, the concentration rates of UCB and UCB-PRP per 1 mL of platelet count were calculated. Furthermore, the concentrations of growth factors (i.e., PDGF-BB, TGF-β1, and VEGF) which were contained in UCB at baseline, 3 months, and 3 years of cryopreservation, were determined quantitatively according to enzyme-linked immunosorbent assay. The following reagents were used according to the manufacturer's package inserts: Quantikine Human PDGF-BB Immunoassay (R&D Systems, Minneapolis, MN, USA) for PDGF-BB; Quantikine Human TGF-β1 Immunoassay (R&D Systems) for TGF-β1; and Quantikine Human VEGF Immunoassay (R&D Systems) for VEGF. A microplate reader, Emax (Molecular Devices, Tokyo, Japan), was used as a gauge.

2.3. Pretreatment of UC-MSCs

Autologous cells, which were obtained from the same infants as those who supplied UCB, were used. UC was treated as per a previously reported method (Baba et al., 2012). Briefly, the Wharton jelly (WJ) was chopped into approximately 5-mm sections. Subsequently, the sections were cultured in Dulbecco's modified Eagle's medium with the addition of 10% UCB autoserum in a 25-cm^2 flask (Sumilon Sumitomo, Tokyo, Japan) at 37 °C and with 5% CO_2. Media were replaced for the first time at 1 week after culture initiation and every 3 days thereafter. The WJ sections were removed at approximately 2 weeks after culture initiation when adhesive spindle-shaped cells emerged. Culture continued until the time when cells became subconfluent (Fig. 3). Subsequently, the cells were detached and collected before suspension in a solution for cell cryopreservation (CELLBANKER 1, Nippon Zenyaku Kogyo, Koriyama, Japan) and storage at −80 °C until the time of use.

2.4. Activities of cryopreserved UCB-PRP on UC-MSCs

MSCs, which had been cryopreserved for 3 years or longer, were thawed at room temperature and put into the 75-cm^2 flask just before cultivation (Matsuo et al., 2008). Culture continued until the time when 80–90% of cells had become subconfluent. Subsequently, the osteoblastic differentiation of UC-MSCs was induced as per a previously reported procedure (Baba et al., 2013). Briefly, the medium was changed to NH OsteoDiff Media (Miltenyi Biotec, Bergisch Gladbach, Germany) once only, with the addition of UCB-PRP at the ratio of 300 mL/mL. Subsequently, the samples of UC-MSCs, which were added with BMP-2 instead of UCB-PRP, were used as positive control; in contrast, the samples of UC-MSCs, which were added with neither UCB-PRP nor BMP-2, were used as negative control. All the samples were stained with Alizarin red.

3. Results

3.1. Volumes of UCB and platelet concentration rates

A total of 9 samples were collected, with volume ranges of 20–50 mL (mean volume: 40.1 mL). The platelet concentration

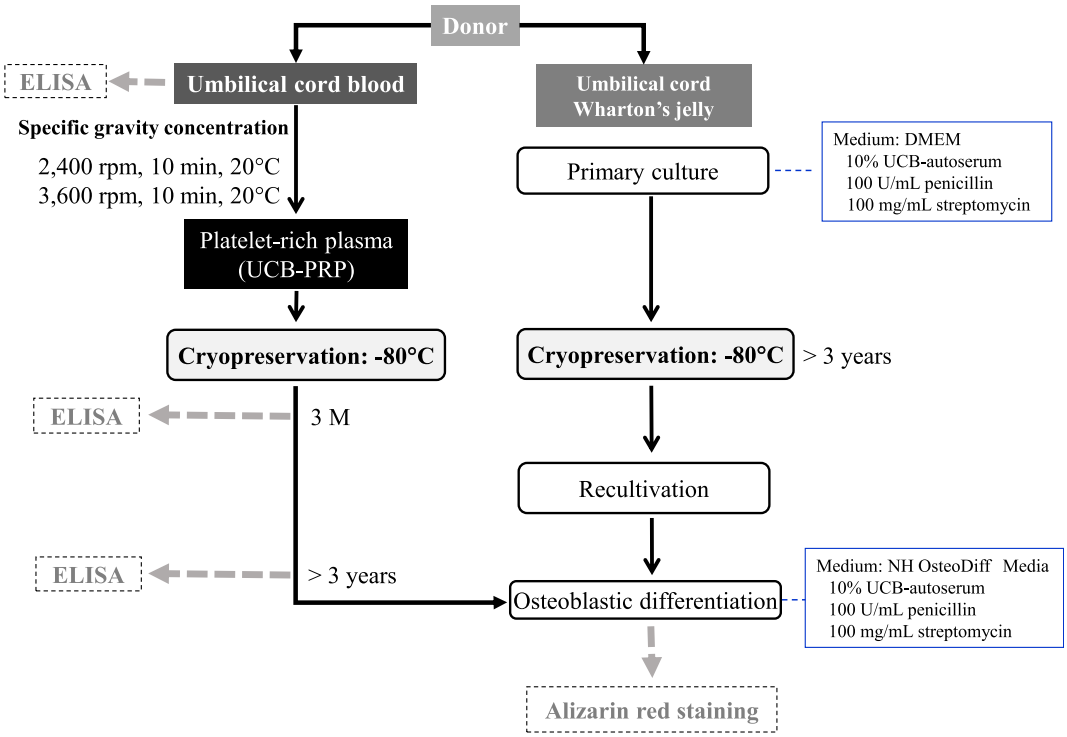

Fig. 1. Overview of the study. ELISA, enzyme-linked immunosorbent assay; UCB-PRP, umbilical cord blood-derived platelet-rich plasma; DMEM, Dulbecco's modified Eagle's medium.

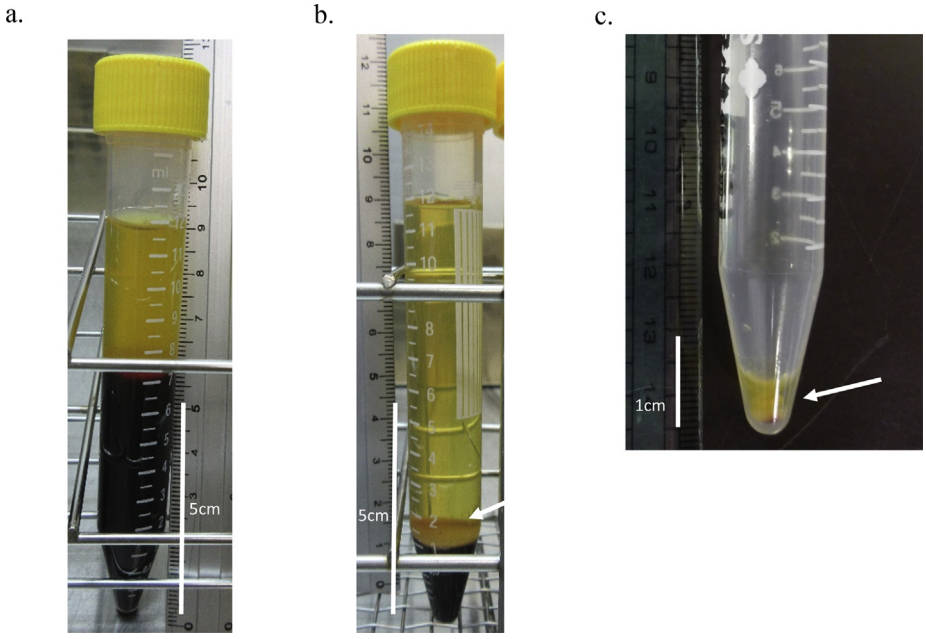

Fig. 2. Centrifugation of the umbilical cord blood. (a) Photograph taken after the first centrifugation. (b) Photograph taken after the second centrifugation (arrow: buffy coat). (c) Umbilical cord blood-derived platelet-rich plasma (arrow).

rates in UCB-PRP varied slightly, with the mean concentration rate of approximately 6.6-fold (Table 2).

3.2. Contents of growth factors in UCB-PRP

At the baseline of cryopreservation, the contents of growth factors in UCB-PRP were as follows: 105–422 pg/mL for PDGF-BB; 2.14–12.0 ng/mL for TGF-β1; and 21–141 pg/mL for VEGF in 2 samples of UCB plasma, but unmeasurable in 2 samples. At 3 months of cryopreservation, the contents of PDGF-BB, TGF-β1, and VEGF in UCB-PRP were 12,200–18,500 pg/mL, 425–566 ng/mL, and 14,900–45,300 pg/mL, respectively. At 3 years of cryopreservation, the contents of PDGF-BB, TGF-β1, and VEGF in UCB-PRP were 113,000–463,000 pg/mL, 890–1,950 ng/mL, and 10,200–77,100 pg/mL, respectively. The contents of growth factors in UCB-PRP cryopreserved for 3 months and 3 years were markedly elevated

Table 1
Allocations of the samples of UCB to experiments.

Sample number	Allocation				
	Concentration rate (platelet count)	GF content in UCB at the baseline of cryopreservation	GF content in UCB at 3 months of cryopreservation	GF content in UCB at 3 years of cryopreservation	Culture
1	Determination				
2	Determination			Determination	
3	Determination	Determination		Determination	
4		Determination	Determination		
5		Determination	Determination		
6		Determination	Determination	Determination	
7					Use
8					Use
9					Use

GF, growth factor; UCB, umbilical cord blood.

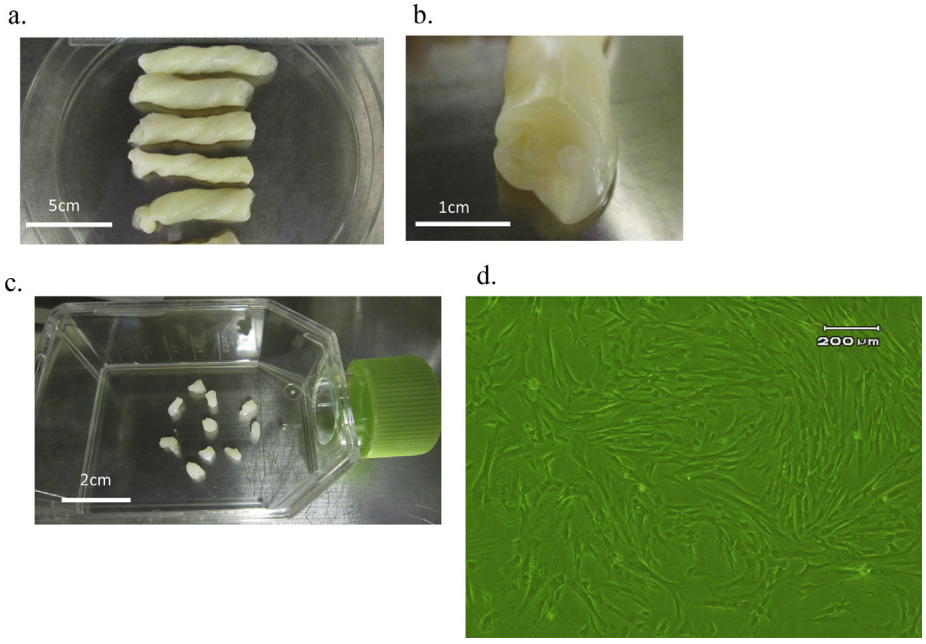

Fig. 3. Preparation of UC-MSCs. (a) Umbilical cords after washing. (b) The cross-section of an umbilical cord after washing. (c) An umbilical cord was chopped, followed by the culture of UC-MCS according to the explant method. (d) Cultured UC-MSCs. UC-MCS, umbilical cord-derived mesenchymal stromal cells.

compared to those found in UCB-PRP at the baseline of cryopreservation. Furthermore, the contents of PDGF-BB and TGF-β1 were greater in the samples of UCB that were added with UCB-PRP cryopreserved for 3 years or longer than those for 3 months (Table 3; Fig. 4).

3.3. Effects of UCB-PRP on the osteoblastic differentiation of UC-MSCs

The samples of UCB that were added with UCB-PRP and those with BMP-2 were stained with Alizarin red, thus suggesting the presence of calcium. On the other hand, the samples of UCB that were not added with growth factors were not stained with Alizarin red (Fig. 5). These findings suggested the osteoblastic induction of UC-MSCs when the samples of UCB were added with UCB-PRP and BMP-2.

4. Discussion

PRP is effective for tissue regeneration through a composite of actions exerted by types of growth factors that are released from concentrated platelets (Dohan et al., 2018; Kawase et al., 2005). Autologous PB-PRP—which has distinguishing features (e.g., autologous in nature, easy preparation, and lack of cellular components)—has been reported to be useful for the reconstruction of hard tissues (e.g., alveolar bone), especially in the specialty of maxillofacial surgery (Kassolis et al., 2000; Marex et al., 1998; Mendonça-Caridad et al., 2006; Suaid et al., 2008; Tomoyasu et al., 2007; Whitman et al., 1997). UCB is currently in clinical use as a congeneic tissue for the treatment of leukemia (Kurtzberg et al., 1996). The presence of UC-MSCs has become well known in recent years, and attention is paid to its usefulness (Kern et al., 2006). On the other hand, a limited number of studies have been published with respect to UCB-PRP. Platelet counts in UCB are considered not to be different from those in adult peripheral blood. However, one cannot tell that the contents and activities of growth factors derived therefrom are identical to those in adult peripheral blood (Lee et al., 2011; Murphy et al., 2012). Furthermore, cryopreservation potentially affects the quality of growth factors. In the present study, therefore, the authors examined the contents and activities of growth factors that were contained in UCB-PRP cryopreserved for 3 years or longer. Bone marrow-derived MSCs (BM-MSCs) are representative among osteogenic cells. In the present study, however, the authors used UC-MSCs as osteogenic cells,

Table 2
Platelet counts in the samples of UCB and platelet concentration rates.

Sample number	Platelet count in whole blood, $10^4/\mu L$	Platelet count in platelet-rich-plasma, $10^4/\mu L$	Platelet concentration rate, %
1	25.6	169.1	6.61
2	33.7	230.8	6.85
3	23.2	149.7	6.42

GF, growth factor; UCB, umbilical cord blood.

Table 3a
Time-course changes in PDGF-BB contents in the samples of UCB.

		Content, pg/mL →	
	272		285,000
Sample	UCB	UCB-PRP at 3 months of cryopreservation	UCB-PRP at 3 years of cryopreservation
2			463,000
3	226		278,000
4	105	18,500	
5	422	14,800	
6	333	12,200	113,000

PDGF-BB, platelet-derived growth factor-BB; UCB, umbilical cord blood; UCB-PRP, umbilical cord blood-derived platelet-rich plasma.

Table 3b
Time-course changes in TGB-β1 contents in the samples of UCB.

		Content, ng/mL →	
	7.24		1,370
Sample	UCB	UCB-PRP at 3 months of cryopreservation	UCB-PRP at 3 years of cryopreservation
2			1,280
3	4.52		1,950
4	2.14	566	
5	10.3	425	
6	12.0	522	890

TGB-β1, transforming growth factor-β1; UCB, umbilical cord blood; UCB-PRP, umbilical cord blood-derived platelet-rich plasma.

Table 3c
Time-course changes in VEGF contents in the samples of UCB.

		Content, pg/mL →	
	81		39,100
Sample	UCB	UCB-PRP at 3 months of cryopreservation	UCB-PRP at 3 years of cryopreservation
2			77,100
3	Unmeasurable		10,200
4	Unmeasurable		10,200
5	21.0	45,300	
6	141	38,800	30,000

VEGF, vascular endothelial growth factor; UCB, umbilical cord blood; UCB-PRP, umbilical cord blood-derived platelet-rich plasma.

because BM-MSCs may differentiate into osteoblasts without osteoblastic differentiation induction (Takeda et al., 2012) and because UC-MSCs also have osteogenic differentiation potential (Matsuo et al., 2008; Sarugaser et al., 2005), apart from the authors' intention of utilizing UC and UCB—autologous tissues that can be obtained noninvasively at the time of birth—for histogenesis.

The clinical effects of PB-PRP are dependent on the platelet concentration rate, and the effective concentration rates have been reported to be 3- to 5-fold (Rebulla et al., 2016; Weibrich et al., 2004). The platelet concentration rates in the present study were about 6-fold, which leads us to consider that UCB-PRP can be expected to be effective clinically.

The contents of growth factors in UCB-PRP cryopreserved for 3 years or longer were much greater than those found in UCB at the baseline of cryopreservation. This finding is considered attributable to the cryopreservation- and thawing-caused release and activation of growth factors that destroyed platelets. The contents of growth factors differ among the samples of UCB collected from individual neonates. However, the contents of growth factors in cryopreserved UCB-PRP that was used in the present study were comparable to those in tissue-engineered PB-PRP (Murphy et al., 2012; Marx, 2004). Unless their biological activities in relation to target cells are sustained, growth factors in cryopreserved UCB-PRP are not utilizable even when their contents are maintained. Furthermore, the effects of UCB-PRP result from the composite interactions of multiple growth factors. Therefore, the effects cannot be simply assessed through the content of a purified single growth factor. Because of this fact, the authors examined the biological activities of UCB-PRP when inducing osteoblastic differentiation of UC-MSCs. Consequently, the samples of UCB in which the osteoblastic differentiation of UC-MSCs was induced by the addition of cryopreserved UCB-PRP and the control to which BMP-2 was added were stained with Alizarin red. The authors conducted Alizarin red staining to examine the ability of cryopreserved UCB-PRP of

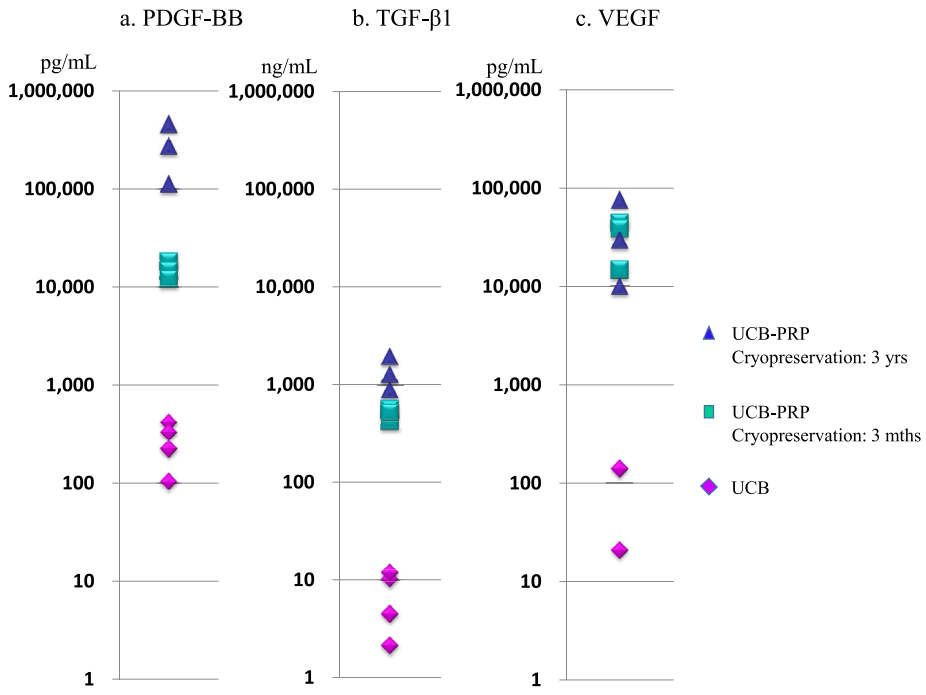

Fig. 4. Contents of growth factors in the umbilical cord blood samples determined at 3 months. and 3 years of cryopreservation. (a) PDGF-BB. (b) TGF-β1. (c) VEGF. PDGF-BB, platelet-derived growth factor-BB; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.

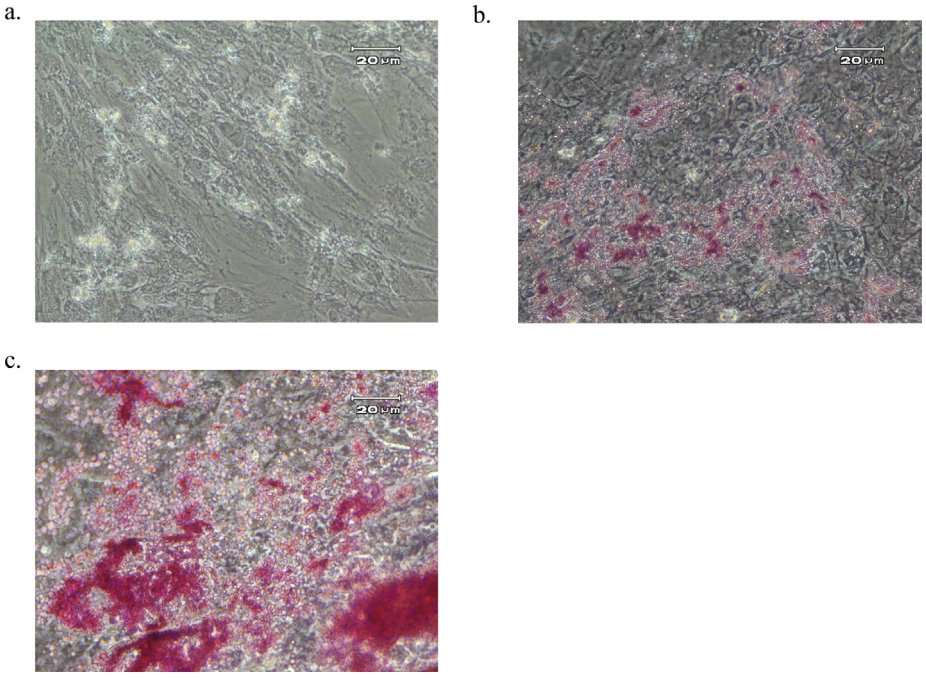

Fig. 5. Photomicrographs showing osteoblastic differentiation-induced, Alizarin red-stained UC-MSCs in the samples of UCB with/without the addition of cryopreserved UCB-PRP or BMP-2. (a) UC-MSCs are not stained in a sample of umbilical cord blood that was added with neither BMP-2 nor cryopreserved UCB-PRP. (b) Extracellular components of a sample added with BMP-2 are stained granularly. (c) Extracellular matrix of a sample added with cryopreserved UCB-PRP is stained granularly. UC-MSCs, umbilical cord-derived mesenchymal stromal cells; UCB, umbilical cord blood; UCB-PRP, umbilical cord blood-derived platelet-rich plasma; BMP-2, bone morphogenetic protein-2.

inducing the osteoblastic differentiation of UC-MSCs in vitro by maximally using samples of limited volume. However, the samples of UCB—to which were added neither UCB-PRP nor BMP-2—were not stained with Alizarin red. These findings led us to consider that the ability of UCB-PRP to induce the osteoblastic differentiation of UC-MSCs was preserved. The authors had a subjective impression that the samples of UCB added with cryopreserved UCB-PRP were more intensively stained with Alizarin red compared to those added with BMP-2. This suggests that cryopreserved UCB-PRP has possibly and advantageously induced the osteoblastic differentiation of UC-MSCs through the composite interactions of multiple growth factors contained therein. It is difficult to consider that

BMP-2 alone is effective for the osteoblastic differentiation of UC-MSCs (Kawase et al., 2005; Pereira et al., 2014). Furthermore, the authors consider that the abovementioned possibility is not incompatible with previous studies (Plachokova et al., 2009; Qi et al., 2015) describing that PB-PRP contains growth factors which accelerate wound healing and hard tissue repair (e.g., PDGF, VEGF, and TGF-β) and is active on the osteogenic potential of BM-MSCs. Unfortunately, the small volume per sample hampered us in determining the degree of osteogenic differentiation by procedures other than Alizarin red staining, a classic method to assess precipitated calcium ions without causing any sample loss. This study is unique and distinguished by the fact that UC-MSCs and PRP originated from the same infants. To determine the degree of osteogenic differentiation in the future, the authors intend to use various osteogenic markers (e.g., ALP, Osx, Runx2, Ocn, and osteopontin) in an *in vitro* study using samples of sufficient volume.

The results from the present study do not allow us to consider that the procedures of cryopreservation and thawing are completely free of any adverse effects on UCB-PRP. In addition, further research will be required because of the limited number of tissue samples used in the present study. Nevertheless, the present study successfully demonstrated that the contents and activities of growth factors are sustained even when using cryopreserved UCB-PRP. The clinical application of UCB-PRP to regenerative medicine requires considerations of ethical issues, safety securement, and therapeutic efficacy. The safety of materials and procedures is a critical challenge to be addressed in clinical application (Ohgushi et al., 2004). The use of autologous tissues as biomaterials for regenerative medicine will allow the avoidance of issues that are caused by the use of xenogeneic or congeneic tissues (e.g., infection, immune response, and ethics). The authors consider that UCB-PRP can be a useful biomaterial in the clinical application to regenerative medicine because of the following advantages: autologous tissue, noninvasive collection, abundant volume of tissue, and simple procedures for preparation. Furthermore, the composite interactions of multiple components that are contained in UCB-PRP potentially and advantageously act on histogenesis in which sorts of cells are involved. The authors intend to examine the utilization of UCB-PRP for co-culture in the future.

The present study has several limitations. First, the number and volume of tissue samples were limited because they were obtained in clinical settings; consequently, the potential activity of UCB-PRP on UCB-MSCs could not be investigated in detail by using various markers for osteogenic differentiation. Second, UC-MSCs and PRP originated from the same infants, resulting in the random allocation of the samples to the respective *in vitro* studies.

5. Conclusion

The authors determined the contents of growth factors (i.e., PDGF-BB, TGF-β1, and VEGF) in UCB-PRP that was cryopreserved for 3 years or longer in an attempt to examine the ability of these growth factors to induce the osteoblastic differentiation of UC-MSCs. The contents were maintained and the ability was sustained. Hence, the potential clinical application to regenerative medicine of UCB-PRP, cryopreserved for 3 years or longer, was suggested.

Acknowledgements

Support was provided by a grant-in-aid for scientific research (C) 16K11376 from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors are grateful to Satoshi Sakima, MD, for his valuable discussions about the manuscript.

References

Baba K, Yamazaki Y, Ikemoto S, Aoyagi K, Takeda A, Uchinuma E: Osteogenic potential of human umbilical cord-derived mesenchymal stromal cells cultured with umbilical cord blood-derived autoserum. J Craniomaxillofac Surg 40(8): 768–772, 2012

Baba K, Yamazaki Y, Ishiguro M, Kumazawa K, Aoyagi K, Ikemoto S, et al: Osteogenic potential of human umbilical cord-derived mesenchymal stromal cells cultured with umbilical cord blood-derived fibrin: a preliminary study. J Craniomaxillofac 41(8): 775–782, 2013

Coffman LG, Choi YJ, McLean K, Allen BL, di Magliano MP, Buckanovich RJ: Human carcinoma-associated mesenchymal stem cells promote ovarian cancer chemotherapy resistance via a BMP4/HH signaling loop. Oncotarget 7(6): 6916–6932, 2016

Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang BS, et al: The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. Platelets 29(2): 171–184, 2018

Hsu WK, Mishra A, Rodeo SR, Fu F, Terry MA, Randelli P, et al: Platelet-rich plasma in orthopaedic applications: evidence-based recommendations for treatment. J Am Acad Orthop Surg 21(12): 739–748, 2013

Kassolis JD, Rosen PS, Reynolds MA: Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. J Periodontol 71(10): 1654–1661, 2000

Kawase T, Okuda K, Saito Y, Yoshie H: In vitro evidence that the biological effects of platelet-rich plasma on periodontal ligament cells is not mediated solely by constituent transforming-growth factor-beta or platelet-derived growth factor. J Periodontol 76(5): 760–767, 2005

Kern S, Eichler H, Stoeve J, Klüter H, Bieback K: Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood or adipose tissue. Stem Cells 24(5): 1294–1301, 2006

Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, et al: Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med 335(3): 157–166, 1996

Lee JY, Nam H, Park YJ, Lee SJ, Chung CP, Han SB, et al: The effects of platelet-rich plasma derived from human umbilical cord blood on the osteogenic differentiation of human dental stem cells. In Vitro Cell Dev Biol Anim 47(2): 157–164, 2011

Marx RE: Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg 62(4): 489–496, 2004

Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR: Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85(6): 638–646, 1998

Matsuo A, Yamazaki Y, Takase C, Aoyagi K, Uchinuma E: Osteogenic potential of human bone marrow-derived mesenchymal stem cells cultured with autologous serum. J Craniofac Surg 19(3): 693–700, 2008

McLean K, Gong Y, Choi Y, Deng N, Yang K, Bai S, et al: Human ovarian carcinoma–associated mesenchymal stem cells regulate cancer stem cells and tumorigenesis via altered BMP production. J Clin Invest 121(8): 3206–3219, 2011

Mendonça-Caridad JJ, Juiz-Lopez P, Rubio-Rodriguez JP: Frontal sinus obliteration and craniofacial reconstruction with platelet rich plasma in a patient with fibrous dysplasia. Int J Oral Maxillofac Surg 35(1): 88–91, 2006

Mishra A, Randelli P, Barr C, Talamonti T, Ragone V, Cabitza P: Platelet-rich plasma and the upper extremity. Hand Clin 28(4): 481–491, 2012

Mooren RE, Hendriks EJ, van den Beucken JJ, Merkx MA, Meijer GJ, Jansen JA, et al: The effect of platelet-rich plasma in vitro on primary cells: rat osteoblast-like cells and human endothelial cells. Tissue Eng A 16(10): 3159–3172, 2010

Murphy MB, Blashki D, Buchanan RM, Yazdi IK, Ferrari M, Simmons PJ, et al: Adult and umbilical cord blood-derived platelet-rich plasma for mesenchymal stem cell proliferation, chemotaxis, and cryo-preservation. Biomaterials 33(21): 5308–5316, 2012

Murphy MS, James HC: Biologic approaches to problems of the hand and wrist. Orthop Clin N Am 48: 343–349, 2017

Ohgushi H, Kitamura S, Kotobuki N, Hirose M, Machida H, Muraki K, et al: Clinical application of marrow mesenchymal stem cells for hard tissue repair. Yonsei Med J 45(Suppl): 61–67, 2004

Pereira T, Ivanova G, Caseiro AR, Barbosa P, Bártolo PJ, Santos JD, et al: MSCs conditioned media and umbilical cord blood plasma metabolomics and composition. PLoS One 9(11): e113769, 2014

Plachokova AS, van den Dolder J, van den Beucken JJ, Jansen JA: Bone regenerative properties of rat, goat and human platelet-rich plasma. Int J Oral Maxillofac Surg 38(8): 861–869, 2009

Qi Y, Niu L, Zhao T, Shi Z, Di T, Feng G, et al: Combining mesenchymal stem cell sheets with platelet-rich plasma gel/calcium phosphate particles: a novel strategy to promote bone regeneration. Stem Cell Res Ther 6: 256, 2015

Rebulla P, Pupella S, Santodirocco M, Greppi N, Villanova I, Buzzi M, et al: Italian Cord Blood Platelet Gel Study Group: multicentre standardisation of a clinical grade procedure for the preparation of allogeneic platelet concentrates from umbilical cord blood. Blood Transfus 14(1): 73–79, 2016

Rodriguez A, Anastassov GE, Lee H, Buchbinder D, Wettan H: Maxillary sinus augmentation with deproteinated bovine bone and platelet rich plasma with simultaneous insertion of endosseous implants. J Oral Maxillofac Surg 61(2): 157–163, 2003

Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE: Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. Stem Cells 23(2): 220–229, 2005

Suaid FF, Carvalho MD, Santamaria MP, Casati MZ, Nociti Jr FH, Sallum AW, et al: Platelet-rich plasma and connective tissue grafts in the treatment of gingival recessions: a histometric study in dogs. J Periodontol 79(5): 888–895, 2008

Takeda A, Yamazaki Y, Baba K, Ishiguro M, Aoyagi K, Ikemoto S, et al: Osteogenic potential of human bone marrow-derived mesenchymal stromal cells cultured in autologous serum: a preliminary study. J Oral Maxillofac Surg 70(8): e469–e476, 2012

Tannoury CA, An HS: Complications with the use of bone morphogenetic protein 2 (BMP-2) in spine surgery. Spine J 14(3): 552–559, 2014

Tomoyasu A, Higashio K, Kanomata K, Goto M, Kodaira K, Serizawa H, et al: Platelet-rich plasma stimulates osteoblastic differentiation in the presence of BMPs. Biochem ophys Res Commun 361(1): 62–67, 2007

van den Dolder J, Mooren R, Vloon AP, Stoelinga PJ, Jansen JA: Platelet-rich plasma: quantification of growth factor levels and the effect on growth and differentiation of rat bone marrow cells. Tissue Eng 12(11): 3067–3073, 2006

Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE: Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone 34(4): 665–671, 2004

Whitman DH, Berry RL, Green DM: Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 55(11): 1294–1299, 1997

Yamada Y, Ito K, Nakamura S, Ueda M, Nagasaka T: Promising cell-based therapy for bone regeneration using stem cells from deciduous teeth, dental pulp, and bone marrow. Cell Transplant 20(7): 1003–1013, 2011

Zara JN, Siu RK, Zhang X, Shen J, Ngo R, Lee M, et al: High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo. Tissue Eng Part A 17(9–10): 1389–1399, 2011