Biochemical and Biophysical Research Communications 497 (2018) 876–882

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Comparison of the bone regeneration ability between stem cells from human exfoliated deciduous teeth, human dental pulp stem cells and human bone marrow mesenchymal stem cells

Kengo Nakajima [a], Ryo Kunimatsu [a, *], Kazuyo Ando [a], Toshinori Ando [b], Yoko Hayashi [c], Takuya Kihara [d], Tomoka Hiraki [a], Yuji Tsuka [a], Takaharu Abe [a], Masato Kaku [a], Hiroki Nikawa [d], Takashi Takata [b], Kazuo Tanne [a], Kotaro Tanimoto [a]

[a] Department of Orthodontics and Craniofacial Developmental Biology, Division of Dental Sciences, Biomedical Sciences Major, Hiroshima University Graduate School of Biomedical & Health Sciences, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8553, Japan
[b] Department of Oral and Maxillofacial Pathobiology, Hiroshima University Graduate School of Biomedical & Health Sciences, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8553, Japan
[c] Analysis Center of Life Science, Natural Science Center for Basic Research and Development, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8553, Japan
[d] Department of Oral Biology and Engineering, Division of Oral Health Sciences, Institute of Biomedical and Health Sciences, Hiroshima University Graduate School, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8553, Japan

ARTICLE INFO

Article history:
Received 14 February 2018
Accepted 18 February 2018

Keywords:
Stem cells from human exfoliated deciduous teeth
Dental pulp stem cells
Bone marrow mesenchymal stem cells
Bone regeneration
Secondary alveolar bone grafting

ABSTRACT

Cleft lip and palate is the most common congenital anomaly in the orofacial region. Autogenous iliac bone graft, in general, has been employed for closing the bone defect at the alveolar cleft. However, such iliac bone graft provides patients with substantial surgical and psychological invasions. Consequently, development of a less invasive method has been highly anticipated. Stem cells from human exfoliated deciduous teeth (SHED) are a major candidate for playing a significant role in tissue engineering and regenerative medicine. The aim of this study was to elucidate the nature of bone regeneration by SHED as compared to that of human dental pulp stem cells (hDPSCs) and bone marrow mesenchymal stem cells (hBMSCs). The stems cells derived from pulp tissues and bone marrow were transplanted with a polylactic-coglycolic acid barrier membrane as a scaffold, for use in bone regeneration in an artificial bone defect of 4 mm in diameter in the calvaria of immunodeficient mice. Three-dimensional analysis using micro CT and histological evaluation were performed. Degree of bone regeneration with SHED relative to the bone defect was almost equivalent to that with hDPSCs and hBMSCs 12 weeks after transplantation. The ratio of new bone formation relative to the pre-created bone defect was not significantly different among groups with SHED, hDPSCs and hBMSCs. In addition, as a result of histological evaluation, SHED produced the largest osteoid and widely distributed collagen fibers compared to hDPSCs and hBMSCs groups. Thus, SHED transplantation exerted bone regeneration ability sufficient for the repair of bone defect. The present study has demonstrated that SHED is one of the best candidate as a cell source for the reconstruction of alveolar cleft due to the bone regeneration ability with less surgical invasion.

1. Introduction

Stem cells derived from various tissues such as bone marrow, adipose tissue, skin, and umbilical cord have been isolated and examined in terms of cell proliferation and differentiation abilities leading to tissue regeneration. Moreover, in recent years, mesenchymal stem cells (MSCs) derived from dental tissues have been studied due to their higher accessibility regarded as the feature.

* Corresponding author. Department of Orthodontics and Craniofacial Developmental Biology, Hiroshima University Graduate School of Biomedical & Health Sciences, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8553, Japan.
E-mail address: ryoukunimatu@hiroshima-u.ac.jp (R. Kunimatsu).

https://doi.org/10.1016/j.bbrc.2018.02.156
0006-291X/

Human dental pulp stem cells (hDPSCs) were first isolated in the year of 2000 [1]. Three years later, stem cells from human exfoliated deciduous teeth (SHED) were isolated [2]. Subsequently, stem cells from periodontal ligament and apical papilla were also isolated and characterized [3,4]. Among them, SHED are derived from the pulp of deciduous teeth, which are clinically and biologically discarded tissues. Thus, SHED are an accessible and promising cell source for tissue regeneration.

Cleft lip and palate is the most common congenital anomaly in the orofacial region. Alveolar bone grafting prior to and during orthodontic treatment are essentially required for most of patients. Autogenous iliac bone grafting is the conventional approach to the closure of bone defects at the alveolar cleft [5]. However, substantial surgical invasion with such complications as hypoesthesia and pain is induced at the donor site after surgery. Furthermore, scarring, hematoma, infection, and fracture of the iliac bone have also been reported [6–9]. Harvesting iliac bone is quite invasive for school-age patients. Consequently, development of a less invasive method has been hopefully anticipated. Toward this goal, we have been developing a certain method available for alveolar bone regeneration for patients with cleft lip and palate using human bone marrow mesenchymal stem cells (hBMSCs). Transplantation of hBMSCs regenerated bone in a dog model of artificial alveolar cleft and an orthodontic tooth movement into the regenerated bone region was achieved [10–12]. However, puncture of the iliac bone is still necessary to obtain cells, and thus, this method still obliges patients an invasion. Therefore, exploring a new cell source for alveolar bone reconstruction is needed.

SHED may be the most promising tool for bone regeneration because of the less invasive procedure for obtaining cells. Application of SHED to regeneration of mineralized tissue have been reported [13–17]. However, comparison of the mineralization ability of SHED, hDPSCs, and hBMSCs has not been fully explored. Moreover, the distribution of cells after transplantation into calvarial bone defects in immunodeficient mice is also still not clear.

The aim of this study was to elucidate the nature of bone regeneration by SHED as compared to that of human dental pulp stem cells and bone marrow mesenchymal stem cells.

2. Materials and methods

Obtaining human dental pulp tissue was approved by the preliminary review board of the epidemiological research committee of Hiroshima University (approval number; E-20). SHED was isolated from upper right primary canine of 11year-old boy who was clinically healthy patients. hDPSCs was isolated from upper right canine of 32-year-old female who was clinically healthy patients and underwent extraction due to orthodontic treatment. Both teeth were obtained with informed consent. SHED and hDPSCs were isolated and cultured as previously described [1,2]. SHED was from hBMSCs (Lonza Walkersville Inc., Walkersville, MD, USA) were purchased and cultured according to the manufacturer's instructions.

2.1. Multipotency evaluation

2.1.1. Osteogenic differentiation

The isolated cells were seeded at a density of 7.4×10^3 cells/well in a 24-well plate. The cells were grown to approximately 60% confluency, and then the media in the wells were replaced with osteogenic differentiation medium to induce osteogenesis. Mineralization was induced on 80% confluent monolayers by addition of DMEM (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum, 100 U/mL Penicillin (Meiji Seika Pharma Co., Ltd., Tokyo, Japan), 100 μg/mL Kanamycin (Meiji Seika Pharma Co., Ltd.), 0.25 μg/mL Amphotericin (MP Biomedicals, Strasbourg, France), 10^{-7} M Dexamethasone (Sigma-Aldrich), 10 mM β-glycerophosphate (Sigma-Aldrich), and 50 μg/mL ascorbate 2-phosphate (MP Biomedicals). Cells were incubated at 37 °C in a 5% CO_2 incubator for 14 days, and the medium was changed every 3 days.

2.1.2. Adipogenic differentiation

When the cells became 100% confluent, the medium was replaced with adipogenic supplement containing hydrocortisone, isobutylmethylxanthine, and indomethacin (R&D Systems, Minneapolis, MN, USA). Cells were incubated at 37 °C in a 5% CO_2 incubator for 14 days, and the medium was changed every 3 days.

2.1.3. Chondrogenic differentiation

Cells were isolated from a monolayer culture and then transferred into tubes to allow formation of three-dimensional (3D) aggregates in medium with chondrogenic supplement containing dexamethasone, ascorbate-phosphate, proline, pyruvate, and recombinant transforming growth factor-β3. Cells were incubated at 37 °C in a 5% CO_2 incubator for 14 days, and the medium was changed every 3 days.

2.1.4. Immunofluorescence staining

Immunofluorescence staining was performed using the human MSC functional identification kit (R&D Systems). After 14-day osteogenic and adipogenic differentiation, the differentiated cells were fixed in 4% paraformaldehyde (Nacalai Tesque Inc., Kyoto, Japan) and rinsed twice with 1% bovine serum albumin (BSA) (Sigma-Aldrich) in phosphate-buffered saline. After 14 days, the pellet culture was fixed in 4% paraformaldehyde and embedded in paraffin. The specimen was cut into sections of 5 μm in thickness with a microtome. The sections were mounted on microscope slides (MATSUNAMI Glass Inc., Ltd., Osaka, Japan). Cells and specimens were then blocked with 0.3% Triton-X (Sigma-Aldrich), 1% BSA, and 10% normal donkey serum (R&D Systems) for 45 min. Then, cells and specimens were incubated with mouse anti-human osteocalcin, aggrecan, or fatty acid-binding protein 4 (FABP4) antibodies (R&D Systems) for 1 h, and incubated for 30 min with a secondary goat anti-mouse immunoglobulin antibody (Southern Biotech, Birmingham, AL, USA). After washing the cells and specimens, the slides were observed with a fluorescence microscope (Biozero BZ8100; KEYENCE, Osaka, Japan).

2.2. Analysis of surface epitopes with flow cytometry

Flow cytometric analysis was performed with unsorted SHED and hDPSCs from the 3rd passage to assess the percentage of cells expressing CD29, CD34, CD44, CD73, CD105, CD271 (Becton Dickinson, San Jose, CA, USA), CD146 (Beckman Coulter, Brea, CA, USA), and STRO-1 (BioLegend Inc., San Diego, CA, USA). Mouse isotype antibodies were served as a control. Ten thousand labeled cells were acquired and analyzed using a FACSVerse flow cytometer (Becton Dickinson) running FLOWJO software (TOMY DIGITAL BIOLOGY Co., Ltd., Tokyo, Japan).

2.3. Cell transplantation

A critical-sized defect of 4.0 mm in diameter was created in the center of the calvaria of immunodeficient mice (BLAB/c-nu: Charles River International Laboratories Inc., Yokohama, Japan) using a trephine bur (IMPLATEX CO., Ltd., Tokyo, Japan) under general anesthesia. These mice were selected to avoid potential immunogenic and graft-rejection responses, because SHED, hDPSCs, and hBMSCs are of human origin. Each cell type was seeded onto a polylactic-*co*-glycolic acid (PLGA) membrane of 4.0 mm in diameter

(GC Inc., Tokyo, Japan) and transplanted into the defect. Four groups, the SHED group, hDPSCs group, hBMSCs group, and a control group without transplantation of cells and membrane, were established. Twenty mice were used with five in each. Twelve weeks after the transplantation, mice were sacrificed for histological analysis. The animal study was performed in accordance with the Ethics Committee of Animal Experiments at Hiroshima University (approval number; A16-107).

2.3.1. Micro-computed tomography (μCT) analysis

Calvariae from these mice were scanned with a μCT scanner (Skyscan1176; Bruker, Kontich, Belgium) immediately after (t_0) and 12 weeks after transplantation (t_1). Scanning was performed with a resolution of 35 μm in the direction parallel to the coronal aspect of the calvariae. ZedView software (LEXI, Tokyo, Japan) was used to make a 3D reconstruction from the micro-radiographic images. RapidForm 2006 (INUS Technology, Seoul, Korea) was also employed for data processing and analysis such as cutting, displacement, and measurement. Filling the bone defect to construct the filled model was performed based on curvature constraint with adjacent faces. Boolean operation of the filled model and the original model was performed to obtain the mass defect volume of the bone defect region using Freeform (SensAble Technologies, Wilmington, MA, USA). The amounts of bone defect at t_0 and new bone formation at t_1 were defined as total volume (TV) and bone volume (BV), respectively. Bone regeneration rate was determined as a percentage of BV/TV using following formula.

Regenerated bone rate (BV/TV) =
Regenerated bone volume at t_1 (BV) */ Bone defect volume at t_0 (TV) × 100

*Regenerated bone volume at t_1 = Bone defect volume at t_0 − Bone defet volume at t_1

2.3.2. Histological evaluation

Hematoxylin and eosin (H&E) staining and Masson's trichrome (MT) staining were performed using standard procedures. MT staining was performed to detect the collagen and osteoid content, stained as light blue. Mature bone and the remaining scaffold observed with H&E staining, and the area of collagen and osteoid observed with MT staining were measured to quantify the percentages of new bone, collagen and osteoid using the BZ-II analysis application software (KEYENCE).

2.3.3. Statistical analysis

All data are presented as the means ± SDs. Statistical differences among groups were analyzed using the Bonferroni method. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Multipotency evaluation

Isolated SHED and hDPSCs were positive for osteocalcin, aggrecan, and FABP4 after 14 days in osteogenic, chondrogenic, and adipogenic culture conditions, respectively (Fig. 1). These data indicated that isolated SHED and hDPSCs possessed the ability to differentiate into osteocytes, chondrocytes, and adipocytes.

3.2. Analysis of surface epitopes with flow cytometry

The expression of CD29, CD34, CD44, CD73, CD105, CD146, CD271, and STRO-1 was assessed on unsorted SHED and hDPSCs after in vitro expansion at passage 3. Flow cytometry analysis revealed that SHED were strongly positive for CD29, CD44, CD73, CD105, and CD146. STRO-1 was expressed in 22.6% of cells. SHED were negative for CD34 and CD271 (Fig. 2a). hDPSCs were strongly positive for CD29, CD44, and CD73. CD105 and CD146 were expressed in 63.9% and 56.7% of hDPSCs, respectively. STRO-1 was expressed in 18.6% of hDPSCs. hDPSCs were negative for CD34 and CD274 (Fig. 2b).

3.3. Cell transplantation

3.3.1. μCT analysis

At t_0, μCT 3D images showed that the defects were almost empty, and no substantial differences were observed among three-

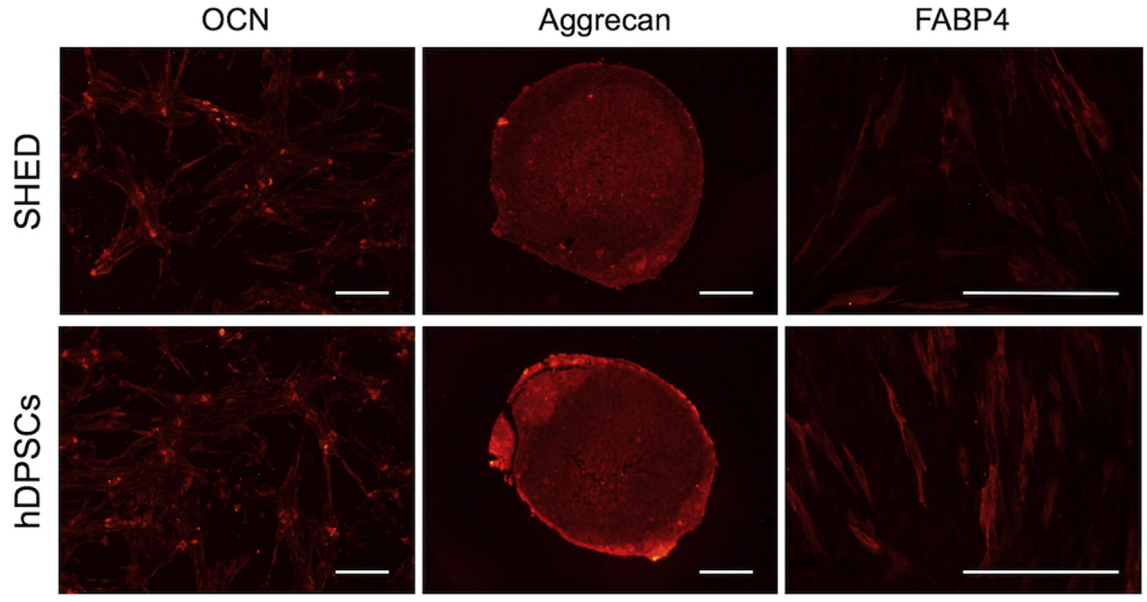

Fig. 1. Multipotency characteristics of isolated SHED and hDPSCs. Isolated SHED showed positive staining for osteocalcin, FABP4, and aggrecan. Isolated hDPSCs showed positive staining for osteocalcin, FABP4, and aggrecan. All scale bars = 300 μm.

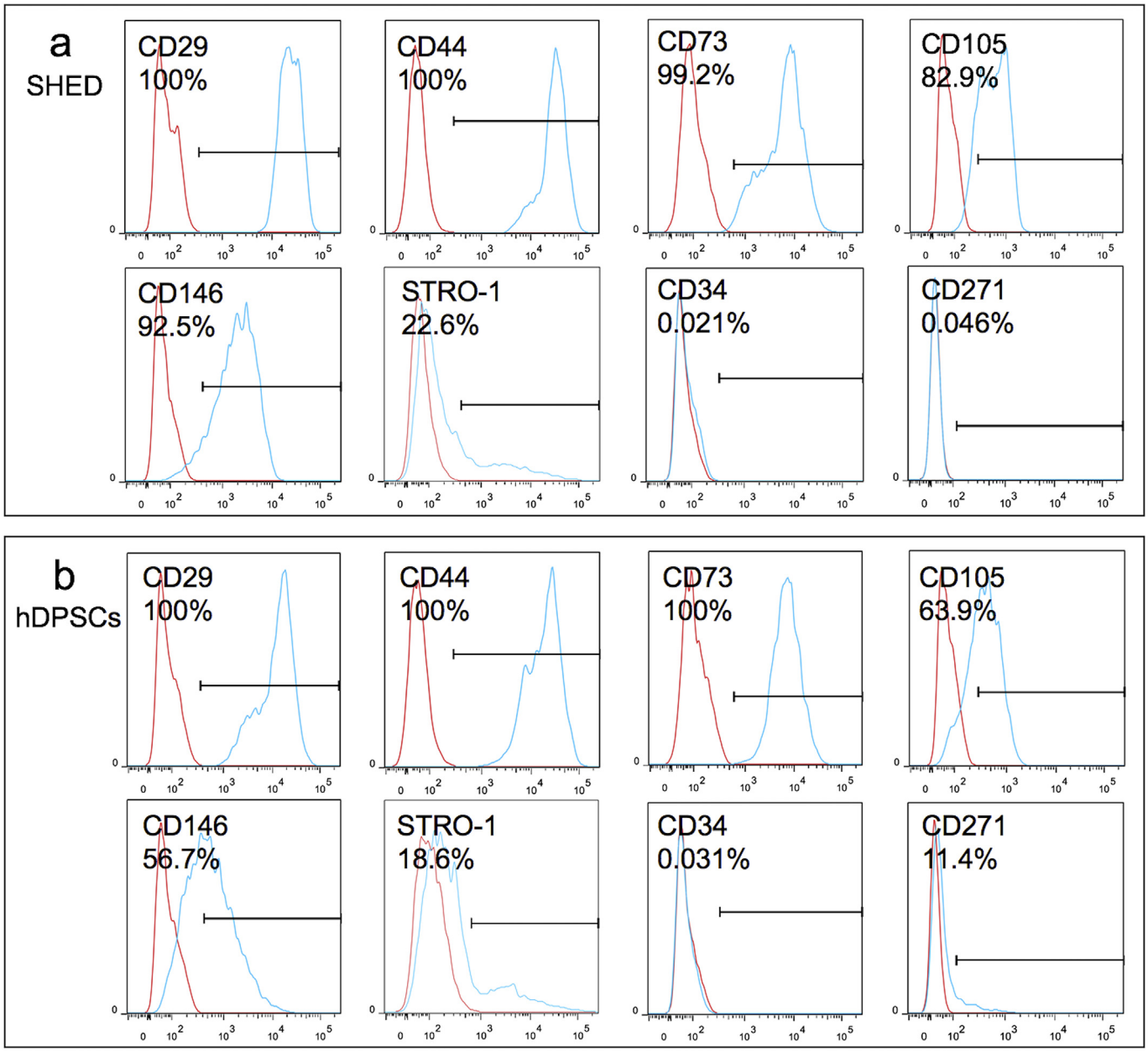

Fig. 2. Flow cytometric analysis of SHED and hDPSCs surface markers. SHED were positive for CD29, CD44, CD73, CD105, CD146, and STRO-1 (Fig. 2a). SHED were negative for CD34 and CD271. hDPSCs were positive for CD29, CD44, CD73, CD105, CD146, and STRO-1 (Fig. 2b). hDPSCs were negative for CD34 and CD271.

group mice. At t_1, μCT 3D images showed a robust amount of new bone formed to repair the defect with transplantation of SHED, hDPSCs, and hBMSCs. The control group showed only a small area of mineralized tissue (Fig. 3a). With respect to the bone regeneration rate, SHED, hDPSCs, and hBMSCs resulted in significantly higher values than the control group ($p < 0.05$) (Fig. 3b), whereas no significant differences were found among the SHED, hDPSCs, and hBMSCs groups.

3.3.2. Histological evaluation

Mature bone is red following MT staining (Fig. 4a). In the control group, H&E staining images showed small new bone area with larger blank area, which represents the remaining scaffold before biodegradation. MT staining showed little expansion of collagen fibers and osteoid. In contrast, in the SHED group, newly formed bone was observed prominently and collagen fibers, osteoid, and a small area of mature lamellate bone were detected along with dura matter in the MT staining images. In the hDPSCs group, a small amount of bone was observed. Meanwhile, in the MT staining images, collagen fibers and osteoid distributed more widely than in the control group. In the hBMSCs group, a small amount of new bone was observed in the center bottom. Cell invasion into the membrane was clearly observed in the central region.

Then, the remaining area occupied by scaffold was quantified in the H&E staining images (Fig. 4b). A large area of the scaffold remained in the control, hBMSCs, hDPSCs, and SHED groups, without any significant differences among all the groups ($p < 0.05$). The collagen and osteoid area was measured in the MT staining images. The SHED group showed a significantly larger collagen and osteoid area than the hDPSCs and control groups ($p < 0.05$), although not significantly different from the hBMSCs group. The hDPSCs group showed a significantly larger area than the control

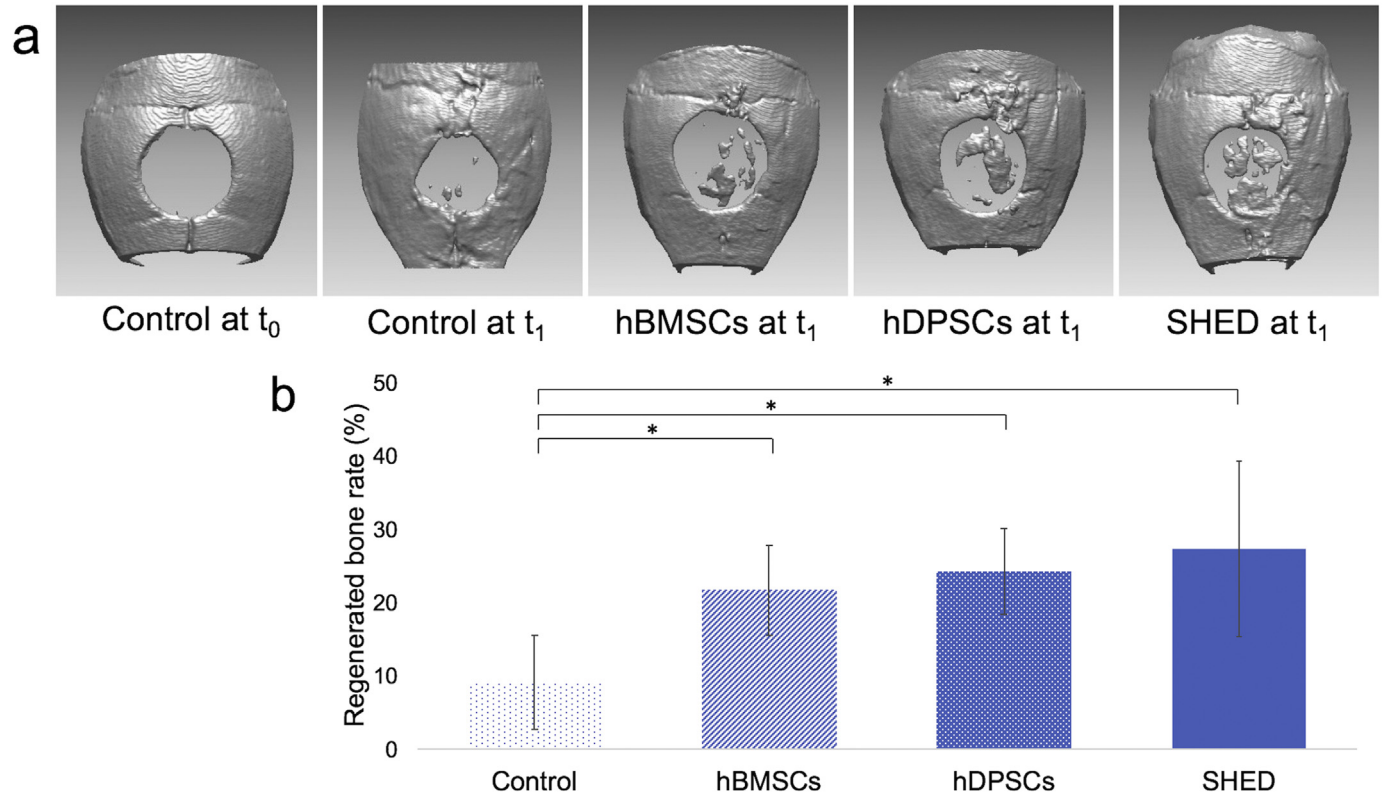

Fig. 3. 3D analysis of regenerated bone from μCT data.
No clear differences were seen among mice at t_0. The control group at t_1 shows that a small amount of mineralized tissue. The hBMSCs group and hDPSCs group showed the same amount of regenerated bone-like tissue. Hence, the SHED group at t_1 revealed that a few mice showed a robust amount of newly formed bone to repair the bone defect (Fig. 3a). The regenerated bone rate of the SHED, hDPSCs, and hBMSCs groups was significantly higher than that of the control group. Although no significant difference was seen among the three transplanted groups, the rate of the SHED group was higher than the rate of the hDPSCs and hBMSCs groups (Fig. 3b). ($n = 5$ for each group, $^*p < 0.05$).

group ($p < 0.05$), but not significantly different from the hBMSCs group.

4. Discussion

Regarding multipotency, in previous studies, SHED and hDPSCs differentiated into osteogenic, chondrogenic, and adipogenic lineages [1,2]. The present results also revealed that both isolated SHED and hDPSCs can differentiate into osteocytes, chondrocytes, and adipocytes. Such differentiation ability, in general, is regarded as one of the minimal criteria to define MSCs.

Specific surface markers of MSCs are CD44, CD73, CD90, CD105, CD271, and STRO-1, whereas CD34, CD45, and HLA-DR are regarded as non-specific or negative markers [18]. However, non-specific marker may happen to be able to identify MSCs, including SHED and hDPSCs. Surface markers of SHED and hDPSCs are still poorly understood. In previous studies regarding surface markers for hDPSCs, the cells were characterized by positive expression of such stromal-associated markers as CD29, CD44, CD73, CD90, and CD105 [19,20]. Meanwhile, the cells are negative for CD14, CD34, and CD45 [21]. Suchánek compared the surface markers between SHED and hDPSCs, and showed that SHED are positive for CD29, CD44, CD73, CD90, CD105, CD117, CD166, and HLA1 similarly to hDPSCs [22]. However, the expression levels of CD71, CD105, CD117, and CD166 were higher in SHED than that in hDPSCs. In this study, CD29, CD44, CD73, CD105, and CD146 were positive in both SHED and hDPSCs, although CD105 and CD146 experienced more prominent expression expressed in SHED (CD105; 82.9%, CD146; 92.9%) than in hDPSCs (CD105; 63.9%, CD146; 56.7%), indicating that stem cells from the pulp of deciduous teeth are immature MSC in nature [23]. CD105 and CD146 are specific markers of endothelial progenitors multipotent MSC, respectively. With a greater differentiation potential, MSCs express a higher percentage of CD146 expression. From such consideration, it is thus concluded that SHED may have a higher potential for differentiation. In previous studies, isolated hDPSCs contained a STRO-1-positive cell population of around 9% [24,25]. Another study revealed that STRO-1-negative cells could be existed following a long time in primary culture before selection [26]. In this study, STRO-1 was expressed in 18.6% of hDPSCs, which is a little higher than in a previous report [27]. STRO-1 was expressed in 22.6% of SHED, which is also a little higher than in previous reports [2,28]. Since SHED and hDPSCs of passage 3 were used in this study, it would be assumed that two kinds of stem cells, SHED and hDPSCs, maintained a higher percentage of STRO-1 expression than in the above-cited previous reports. From these results, multipotency characteristics and specific surface markers can be demonstrated for both of SHED and hDPSCs.

Many *in vivo* studies have reported the suitability of SHED and hDPSCs for bone regeneration in artificially-created defects on the calvaria. de Mandonca et al. showed that SHED and a collagen membrane induce new bone formation in calvarial bone defects with formation of lamellar bone in rats [14]. Riccio et al. reported that fibroin scaffolds provide an optimal microenvironment for osteogenic differentiation and bone formation using hDPSCs [15]. The osteogenic ability of both SHED and hDPSCs was superior to that of hBMSCs. The results in our current study showed that transplantation of PLGA membrane with SHED or hDPSCs induced the same amount of new bone formation in the calvarial bone defect and that the amount of new bone was also approximately the same as with hBMSCs transplantation. SHED transplantation resulted in the highest bone regeneration rate, although no significant differences were found among SHED, hDPSCs, and hBMSCs.

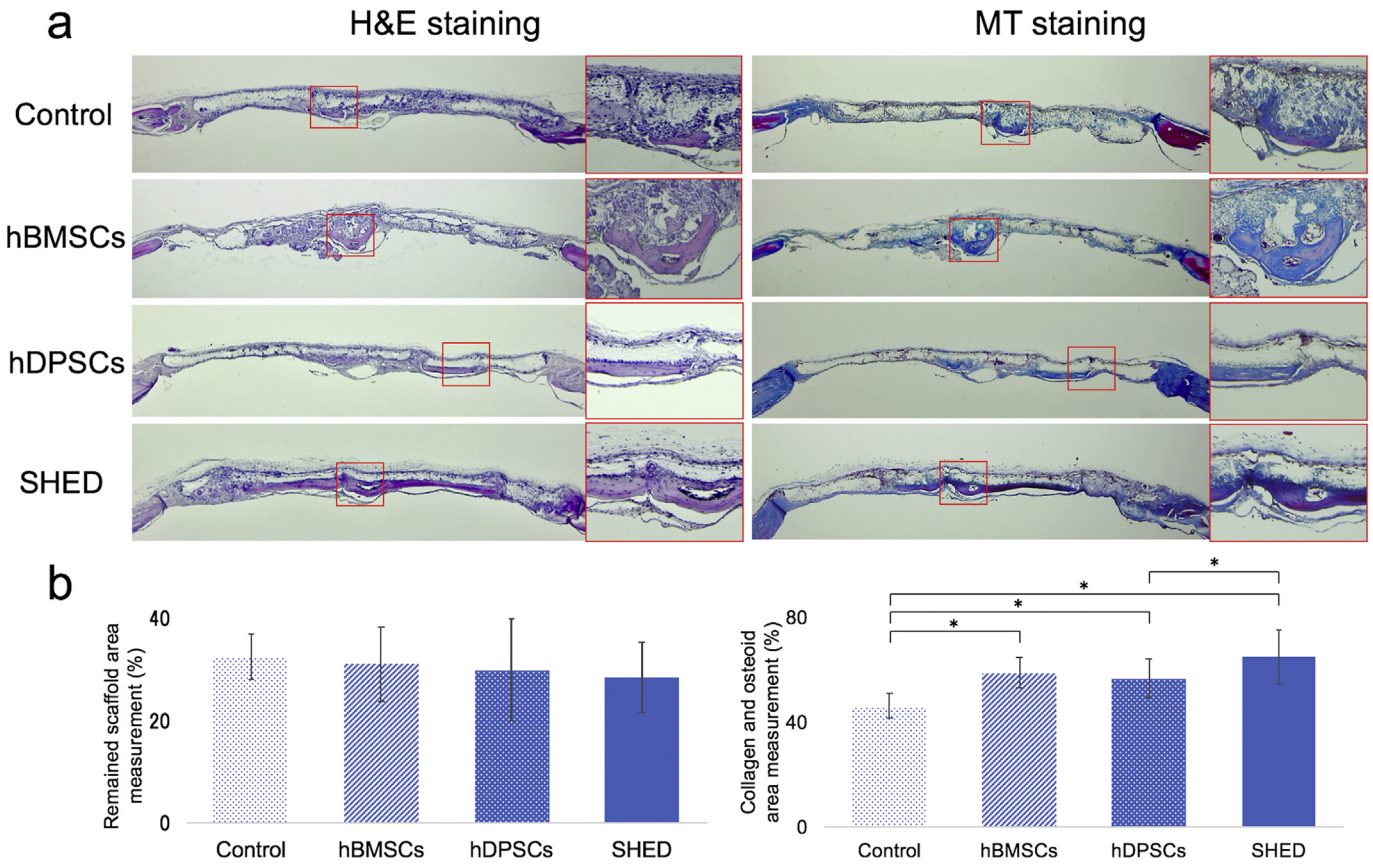

Fig. 4. Histological evaluation 12 weeks after cell transplantation. H&E staining in the control group showed less new bone area and more blank areas, which represent the remaining scaffold before metabolism (Fig. 4a). MT staining showed a little expansion of collagen fibers and osteoid. In the hBMSCs group, a small amount of new bone was observed at the central bottom. Newly formed bone was observed more prominently in the main specimen of the SHED group. Collagen fibers, osteoid, and mature lamellate bone were observed along with dura matter. Measurement of the remaining scaffold area with H&E staining showed no significant difference among all groups ($n = 15$ for each group, $p < 0.05$) (Fig. 4b). Measurement of collagen and osteoid area with MT staining showed that the SHED group had the largest area among all groups. Significant differences were found other than between hBMSCs and hDPSCs group and between hDPSCs and SHED group ($n = 15$ for each group, $^{*}p < 0.05$).

In the quantitative histological analysis, although we found no significant differences among the groups in remaining scaffold region, SHED had the highest percentage of collagen and osteoid area among the groups. These results indicate that SHED transplantation can more promote bone regeneration and repair bone defects compared with others. Hence, according to the degree of remaining scaffold, it may be confirmed that cell transplantation may not effect biodegradaion of the PLGA membrane. The PLGA membrane was used in the present study for the following reasons. The membrane is a bioabsorbable material and has already been approved for clinical application. Furthermore, cells can proliferate on the membrane and prevent soft tissue invasion into a bone defect [29]. In addition, the membrane has sufficient strength to maintain the three-dimensional shape until the bone is regenerated. Moreover, human MSCs have an ability to differentiate into both bone and cartilaginous tissues in a 3D collagen scaffold, which is biocompatible and can be degraded under an *in vivo* condition [30], but not sufficiently strong to maintain the 3D shape in the alveolar cleft after its transplantation until bone regeneration is achieved. HAP and β-TCP, other candidate scaffolds for bone reconstruction, have been examined for bone regeneration [31,32]. However, high possibility in the induction of infection and difficulty in tooth movement into bone regeneration area after transplantation still remain as problems to be solved [33]. Our research group has reported that PLGA membranes are clinically applicable for guided bone regeneration (GBR) for an alveolar bone defect in a patient with a relatively small alveolar cleft [34]. After the GBR procedure, a bone bridge was formed at the alveolar bone defect, and the alveolar bone height and width increased during the postoperative observation period. It is also reported in our studies that transplantation of MSCs with carbonated hydroxyapatite (CAP) into artificial alveolar clefts in dog model showed good potential as an alternative treatment modality [10,11]. Appropriate scaffold for alveolar bone regeneration requires more extensive and further examination.

Since the development, autogenous alveolar bone grafting has long been performed successfully for bone reconstruction. A fatal shortcoming is the surgical invasion for sampling iliac bone in growing patients. Moreover, pain, hypoesthesia, and prolonged hospitalization are substantial demerits. Autogenous bone transplants are inadequate for large defects and involve such risks as donor site morbidity, graft failure, immunological rejection, and infection [35,36]. Some clinical trials for closure of alveolar cleft defects with hBMSCs have been reported [37–41]. The results reveal that the postoperative pain in donor site is less intensity and its frequency compared with the traditional iliac bone graft technique. On the other hand, the bone augmentation with the hBMSCs application has not demonstrated a significant increase. Based on the previous results, cell transplantation to regenerate bone shows good potential over current treatment options. In the present study, SHED transplantation revealed good results in terms of successful bone regeneration to repair an artificially-created bone defect on the calvaria of immunodeficient mice. SHED may thus become one of the best candidates as a cell source for the treatment of alveolar

cleft in CLP patients due to the less surgical and psychological invasions in nature during sampling and transplanting the cells. For bone regeneration in adult CLP patients with no deciduous teeth, hDPSCs could be derived from wisdom teeth or supernumerary teeth. It is hopefully anticipated that SHED cryopreservation may be become a useful tool to maintain the resource of MSCs for clinical applications [42–44]. In near future, more extensive studies will be of a great importance to achieve such advanced technology.

5. Conclusions

This study has demonstrated the followings: (1) isolated SHED and hDPSCs share features with MSCs, (2) transplantation of SHED and hDPSCs induced new bone formation, (3) transplantation of SHED and hDPSCs resulted in approximately the same amount of new bone formation as hBMSCs transplantation. These results indicate that SHED may be one of the best cell source candidates for reconstructing an alveolar cleft due to the less invasions during sampling the cells.

Conflicts of interest

All authors in this manuscripts have no conflicts of interest.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science, Tokyo, Japan (KAKENHI Grant Numbers 16K11788 and 17K17327). The authors would like to thank Professor Koichi Kato, Department of Biomaterials, Hiroshima University Graduate School of Biomedical & Health Sciences, for kindly providing valuable technical support and advice.

References

[1] S. Gronthos, M. Mankani, J. Brahim, et al., Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 13625–13630.
[2] M. Miura, S. Gronthos, M. Zhao, et al., SHED: stem cells from human exfoliated deciduous teeth, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 5807–5812.
[3] B.M. Seo, M. Miura, S. Gronthos, et al., Investigation of multipotent postnatal stem cells from human periodontal ligament, Lancet 364 (2004) 149–155.
[4] W. Sonoyama, Y. Liu, D. Fang, et al., Mesenchymal stem cell-mediated functional tooth regeneration in swine, PLoS One 1 (2006) e79.
[5] A.S. Murthy, J.A. Lehman, Evaluation of alveolar bone grafting: a survey of ACPA teams, Cleft Palate Craniofac. J. 42 (2006) 99–101.
[6] S.W. Laurie, L.B. Kaban, J.B. Mulliken, et al., Donor-site morbidity after harvesting rib and iliac bone, Plast. Reconstr. Surg. 73 (1984) 933–938.
[7] L.T. Kurz, S.R. Garfin, R.E. Booth Jr., Harvesting autogenous iliac bone grafts: a review of complications and techniques, Spine 14 (1989) 1324–1331.
[8] E.M. Younger, M.W. Chapman, Morbidity at bone graft donor site, J. Orthop. Trauma 3 (1989) 192–195.
[9] C. Myeroff, M. Archdeacon, Current concepts review. Autogenous bone graft: donor sites and techniques, J. Bone Joint Surg. 93 (2011) 2227–2236.
[10] M. Yoshioka, K. Tanimoto, Y. Tanne, et al., Bone regeneration in artificial jaw cleft by use of carbonated hydroxyapatite particles and mesenchymal stem cells derived from iliac bone, Int, J. Dent. (2012) 352510.
[11] K. Tanimoto, K. Sumi, M. Yoshioka, et al., Experimental tooth movement into new bone area regenerated by use of bone marrow-derived mesenchymal stem cells, Cleft Palate Craniofac. J. 52 (2015) 386–394.
[12] K. Sumi, T. Abe, R. Kunimatsu, et al., The effect of mesenchymal stem cells on chemotaxis of osteoclast precursor cells, J. Oral Sci. (2017) in press.
[13] B.M. Seo, W. Sonoyama, T. Yamaza, et al., SHED repair critical-size calvarial defects in mice, Oral Dis. 14 (2008) 428–430.
[14] A. de Mendonça Costa, D.F. Bueno, M.T. Martins, et al., Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells, J. Craniofac. Surg. 19 (2008) 204–210.
[15] M. Riccio, T. Maraldi, A. Pisciotta, et al., Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells, Tissue Eng. 18 (2012) 1006–1013.
[16] K. Sakai, A. Yamamoto, K. Matsubara, et al., Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms, J. Clin. Invest. 122 (2012) 80–90.
[17] C. Gandia, A. Armiñan, J.M. García-Verdugo, et al., Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction, Stem Cell. 26 (2008) 638–645.
[18] A. Uccelli, L. Moretta, V. Pistoia, Mesenchymal stem cells in health and disease, Nat. Rev. Immunol. 8 (2008) 726–736.
[19] Y.Y. Jo, H.J. Lee, S.Y. Kook, et al., Isolation and characterization of postnatal stem cells from human dental tissues, Tissue Eng. 13 (2007) 767–773.
[20] A. Pivoriuūnas, A. Surovas, V. Borutinskaite, et al., Proteomic analysis of stromal cells derived from the dental pulp of human exfoliated deciduous teeth, Stem Cell. 19 (2010) 1081–1093.
[21] G. Laino, R. d'Aquino, A. Graziano, et al., A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB), J. Bone Miner. Res. 20 (2005) 1394–1402.
[22] J. Suchánek, B. Visek, T. Soukup, et al., Stem cells from human exfoliated deciduous teeth-Isolation, long term cultivation and phenotypical analysis, Acta Med. 53 (2010) 93–99.
[23] I. Kerkis, A. Kerkis, D. Dozortsev, et al., Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers, Cells Tissues Organs 184 (2006) 105–116.
[24] P.R. Cullis, A. Chonn, Recent advances in liposome technologies and their applications for systemic gene delivery, Adv. Drug Deliv. Rev. 30 (1998) 73–83.
[25] I. Roy, S. Mitra, A. Maitra, et al., Calcium phosphate nanoparticles as novel non-viral
vectors for targeted gene delivery, Int. J. Pharm. 250 (2003) 25–33.
.
[26] P.J. Simmons, B. Torok-Storb, Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1, Blood 78 (1991) 55–62.
[27] G.T. Huang, S. Gronthos, S. Shi, Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine, J. Dent. Res. 88 (2009) 792–806.
[28] X. Wang, X.J. Sha, G.H. Li, et al., Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells, Arch. Oral Biol. 57 (2012) 1231–1240.
[29] T. Kaneko, Development of GC membrane and investigation for tissue-engineered scaffold, J. Jpn. Assoc. Regen 6 (2009) 90–98.
[30] K. Tanimoto, K. Honda, N. Tanaka, et al., Multidifferentiation potential of mesenchymal stem cells in three-dimensional collagen gel cultures, J. Biomed. Mater. Res. 75 (2005) 733–741.
[31] K. Ogata, N. Nakamura, A. Suzuki, et al., Clinical evaluation of secondary grafting of a mixture of Hydroxyapatite and autogenous particulate cancellous bone and marrow in the alveolar cleft, J. Jpn. Cleft Palate Assoc. 25 (2000) 215–223.
[32] M.Z, S. Hossain, K.Tanne Kyomen, Biologic responses of autogenous bone and beta-tricalcium phosphate ceramics transplanted into bone defects to orthodontic forces, Cleft Palate Craniofac. J. 33 (1996) 277–283.
[33] S.E. Feinberg, M. Vitt, Effect of calcium phosphate ceramic implants on tooth eruption, J. Oral Maxillofac. Surg. 46 (1988) 124–127.
[34] M. Yuki, T. Kawata, Y. Miyamoto, et al., A cleft lip and alveolus patient treated with guided bone regeneration (GBR) technique for the alveolar bone defect, J. Jpn. Cleft Palate Assoc. 28 (2003) 250–260.
[35] P.N. Manson, W.A. Crawley, J.E. Hoopes, Frontal cranioplasty: risk factors and choice of cranial vault reconstructive material, Plast. Reconstr. Surg. 77 (1986) 888–904.
[36] C. Dahlin, P. Alberius, A. Linde, Osteopromotion for cranioplasty. An experimental study in rats using a membrane technique, J. Neurosurg. 74 (1991) 487–491.
[37] H. Hibi, Y. Yamada, M. Ueda, et al., Alveolar cleft osteoplasty using tissue-engineered osteogenic material, Int. J. Oral Maxillofac. Surg. 35 (2006) 551–555.
[38] M. Gimbel, R.K. Ashley, M. Sisodia, et al., Repair of alveolar cleft defects: reduced morbidity with bone marrow stem cells in a resorbable matrix, J. Craniofac. Surg. 18 (2007) 895–901.
[39] H. Behnia, A. Khojasteh, M. Soleimani, et al., Secondary repair of alveolar clefts using human mesenchymal stem cells, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 108 (2009) e1.
[40] R. d'Aquino, A. De Rosa, V. Lanza, et al., Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes, Eur. Cell. Mater. 18 (2009) 75–83.
[41] H. Behnia, A. Khojasteh, M. Soleimani, et al., Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: a preliminary report, J. Cranio-Maxillo-Fac. Surg. 40 (2012) 2–7.
[42] W. Zhang, X.F. Walboomers, S. Shi, et al., Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation, Tissue Eng. 12 (2006) 2813–2823.
[43] G. Papaccio, A. Graziano, R. d'aquino, et al., Long-Term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: a cell source for tissue repair, J. Cell. Physiol. 208 (2006) 319–325.
[44] H.S. Lee, M. Jeon, S.O. Kim, et al., Characteristics of stem cells from human exfoliated deciduous teeth (SHED) from intact cryopreserved deciduous teeth, Cryobiology 71 (2015) 374–383.