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Research article

Osteogenic potency of dental stem cell-composite scaffolds in an animal cleft palate model

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ABSTRACT

Objective: To evaluate the osteogenic potency of stem cells isolated from human exfoliated deciduous teeth (SHED) in polycaprolactone with gelatin surface modification (PCL-GE) and poly (lactic-*co*-glycolic acid)-bioactive glass composite (PLGA-bioactive glass (BG)) scaffolds after implantation in a rat cleft model.

Methods: Cleft palate-like lesions were induced in Sprague-Dawley rats by extracting the right maxillary first molars and drilling the intact alveolar bone. Rats were then divided into five groups: Control, PCL-GE, PCL-GE-SHED, PLGA-BG, and PLGA-BG-SHED, and received corresponding composite scaffolds with/without SHED at the extraction site. Tissue samples were collected at 2, 3, and 6 months post-implantation (4 rats per group). Gross and histological analyses were conducted to assess osteoid or bone formation. Immunohistochemistry for osteocalcin and human mitochondria was performed to evaluate bone components and human stem cell viability in the tissue.

Results: Bone tissue formation was observed in the PCL-GE and PLGA-BG groups compared to the control, where no bone formation occurred. PLGA-BG scaffolds demonstrated greater bone regeneration potential than PCL-GE over 2–6 months. Additionally, scaffolds with SHED accelerated bone formation compared to scaffolds alone. Osteocalcin expression was detected in all rats, and positive immunoreactivity for human mitochondria was observed in the regenerated bone tissue with PCL-GE-SHED and PLGA-BG-SHED.

Conclusion: PCL-GE and PLGA-BG composite scaffolds effectively repaired and regenerated bone tissue in rat cleft palate defects. Moreover, scaffolds supplemented with SHED exhibited enhanced osteogenic potency.

Clinical significance: PCL-GE and PLGA-BG scaffolds, augmented with SHED, emerge as promising biomaterial candidates for addressing cleft repair and advancing bone tissue engineering endeavors.

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1. Introduction

Orofacial clefts, including a cleft lip with or without cleft palate, are one of the most common congenital abnormalities of the head and neck [1–3]. Their causes are multifactorial and complex, including genetics, environmental factors, teratogen exposure, and parental age [2,4,5]. A cleft palate, characterized by the incomplete fusion of the palate, significantly impacts the health and quality of life of infant patients. It leads to various challenges such as speech pathology, feeding difficulties, ear-related issues, psychological concerns, and long-term financial burdens. Furthermore, cleft palates increase morbidity and mortality rates at all ages [2,6].

Generally, the predominant treatment for cleft palate is surgical repair [1,2,6–8]. However, conventional surgical procedures, such as using a primary bone graft, have negative adverse effects, including invasive procedures for obtaining the bone graft, donor site morbidity, increased operative time, induction of malignancies, and graft loss [3,9,10]. Thus, a combination of biomaterials as tissue scaffolds with various stem cells is a widely used alternative for surgical treatment [6]. Biomaterial scaffolds should have physical stability, biocompatibility, promote neovascularization and stem cell recruitment, as well as show progressive resorption during replacement by host tissue, and promotion of tissue regeneration [5]. Currently, many studies focus on synthetic polymer scaffolds, including polycaprolactone (PCL) and poly (lactic-*co*-glycolic acid, PLGA) [11–13]. Both are synthetic biodegradable polyesters fabricated for biomedical materials. PCL has excellent mechanical properties with high strength and elasticity and is nontoxic, biodegradable, tissue compatible [14,15], and nonimmunogenic [13]. PCL allows cell adhesion, proliferation, and differentiation [11, 14,16,17]. On the other hand, the natural polymer gelatin increases cellular activity, as highlighted by the increased proliferation of human mesenchymal stem cells on the surface of the modified polyurethane urea [18]. Moreover, gelatin immobilization results in higher osteogenic differentiation than bare polycaprolactone [19]. Similar to PCL, PLGA is a favorite biodegradable polymer with good mechanical and biological properties [20], showing good cell attachment and proliferation [21,22]. In combination with bioactive glass (BG), composed of SiO_2, Ca^{2+}, Na^{+} and PO_4^{3-}, PLGA enhances osteoinductivity and encourages bone formation, which is why this combination was utilized in bone grafting studies [23–26].

Stem cells from human exfoliated deciduous teeth (SHEDs) are interesting as a stem cell source for regenerative medicine [27–29]. They are obtained from healthy human dental pulp tissue and are multipotent [29,30]. SHEDs are considered one of the best candidates for studying bone formation as they do not have limited potency to differentiate into osteoblasts and bony tissue [31,32].

From the hypothesis of the study that proposed the new combination usage of the SHED and scaffolds to promote the bone tissue reconstruction in the cleft mimic model. The current study aimed to evaluate the osteogenic potency of SHED in polycaprolactone with gelatin surface modification (PCL-GE) and poly (lactic-*co*-glycolic acid)-bioactive glass (PLGA-BG) composite scaffolds after implantation in a rat cleft model. The results of the study would provide the information about the application of SHED and current types of scaffolds which is the novel techniques and materials in the cleft patient treatment.

2. Materials and methods

2.1. Preparation of a scaffold with polycaprolactone and gelatin surface modification

The gelatin surface modification of electrospun polycaprolactone fibers (PCL-GE) was produced as previously described [16–18, 33]. Briefly, a 17 % w/v PCL solution was obtained by dissolving PCL pellets (molecular weight from 70,000 to 90,000 g/mol, Sigma-Aldrich, St. Louis, MO, USA) in chloroform:methyl alcohol (3:1 by volume). The PCL solution was transferred to a 25-mL glass syringe with a 0.9-mm metal nozzle. A high-voltage power supply was used to charge the loaded solution by connecting an emitting electrode to the nozzle. Aluminum foil was used as a fiber collector and placed 15 cm from the nozzle tip. Then, the electrospun PCL fiber scaffold was produced by applying a 20-kV electrical potential. The flow rate of the PCL solution was set at 5 mL/h and the sample was collected for 2 h at room temperature. Subsequently, the surface of the PCL scaffold underwent modification by immersing it in ethylenediamine (Loba Chemie Ltd., Mumbai, IN) for 1 h to introduce an amine group. After aminolysis, the scaffold was immersed in a 5 % w/v glutaraldehyde solution (Thermo Fisher Scientific, Waltham, MA, USA) for 3 h. Subsequently, the scaffold was bound with gelatin by immersion in a 5 g/L gelatin solution (Thermo Fisher Scientific) at 4 °C for 24 h. Then, the free aldehyde was blocked by immersion in 3 g/L glycine (Amresco, Solon, OH, USA) for 1 h. The products were called PCL-GE scaffolds and maintained at 4 °C until use.

2.2. Preparation of poly (lactic-co-glycolic acid)-bioactive glass composite scaffolds

The poly (lactic-*co*-glycolic acid)-bioactive glass (PLGA-BG) scaffolds were fabricated as previously described [22,34]. The bioactive glass (BG) was synthesized by the sol–gel method. Briefly, tetraethyl orthosilicate (Thermo Fisher Scientific) was mixed with a nitric solution (HNO_3^-; Carlo Erba, Val de Reuil, FR) and stirred for 1 h. Then, triethyl phosphate [$(C_2H_5)_3PO_4$; Thermo Fisher Scientific], calcium nitrate tetrahydrate ($Ca(NO_3)_2$; Carlo Erba), and sodium nitrate ($NaNO_3$; Carlo Erba) were individually added to the solution and stirred for 45 min. Sol–gel formation was observed after storage for five days at room temperature. The gel was then aged at 70 °C for one day and dried at 120 °C for another day. Finally, the gel was stabilized and nitrate residues were removed from the dried sample at 850 °C for one day. Subsequently, the salt-leaching technique was used to prepare the composite scaffolds. To prepare the PLGA with 10 % BG composite scaffold, 0.10 g of bioactive glass, 1 g of PLGA polymer (Changchun Foliaplast Bio-Tech Co., Ltd, Jilin, CN), 15 g of sodium chloride (Sigma-Aldrich), and 10 mL of chloroform (Sigma-Aldrich) were mixed under magnetic stirring at room temperature. The complete mixture was cast into a mold, and the thickness controlled at 5 mm. Cast scaffolds were dried at room temperature for three days, and sodium chloride was removed by membrane dialysis with deionized water.

2.3. SHED preparation

All experiments were approved by the Ethical Committee on Human Rights Related to Human Experimentation of the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University (MU-DT/PY-IRB 2014/041.2110). We isolated, cultured, and characterized SHEDs according to previous studies [22,35,36]. Briefly, pulp tissues obtained from the deciduous teeth of healthy donors were submerged in a cell culture medium containing Dulbecco's modified Eagle's medium (DMEM, HyClone, Fisher Scientific, Loughborough, UK), 10 % fetal bovine serum (FBS, Biochrome, Berlin, GY), 100 U/mL Penicillin (Gibco, Thermo Fisher Scientific), and 100 μm/mL Streptomycin (Gibco). The cultures were maintained at 37 °C with 5 % CO_2, with the medium changed every other day until the outgrowth cells reached 80 % confluence. Cells were then sub-cultured or harvested using 0.05 % Trypsin/EDTA (Gibco). Characterization showed cells were positive for mesenchymal stem cell surface markers (CD73, CD105, CD146) and negative for hematopoietic stem cell antigen (CD34). Additionally, the cells demonstrated multipotential differentiation into osteoblasts, chondrocytes, and adipocytes when assessed using specific media.

Then, the composite scaffolds were placed onto a 96-well plate and 10 μL of SHED (passages 6–9) suspension containing 2×10^4 cells was added to each well. The SHEDs growing in the scaffolds were incubated in an Advance STEM osteogenic differentiate medium kit (GE Healthcare, West Milwaukee, WI, USA) for four days before transplantation.

2.4. Animal cleft palate model

The animal protocol was reviewed and approved by the Faculty of Science, Mahidol University-Institutional Animal Care and Use Committee (MUSC-ACUC) with approval number MUSC60-034-384. Sixty 7-week-old male Sprague–Dawley rats were obtained from Nomura Siam International Co., Ltd, Bangkok, Thailand. The rats were acclimatized for a week before beginning with the experiment. The cleft palate mimicking lesion was induced by extraction of the right permanent maxillary first molar. Briefly, rats were anesthetized with xylazine (Rompun®, Bayer, Leverkusen, DE) and maintained by isoflurane inhalation (Aerrane®, Baxter Healthcare, SG) during the surgical procedure. The rat's oral cavity was disinfected using 0.12 % chlorhexidine (Osoth Inter Laboratory, Bangkok, THA) and sterile normal saline. Then, to approach the tooth, the gingival tissue adjacent to the right maxillary first molar was incised using a surgical blade (Fig. 1A). The entire molar was extracted using extraction forceps and the intact alveolar bone was drilled using dental burs (Fig. 1B). Next, the scaffold was placed at the extraction site (Fig. 1C) before suturing the incised gingival tissue to stabilize the implanted scaffold (Fig. 1D).

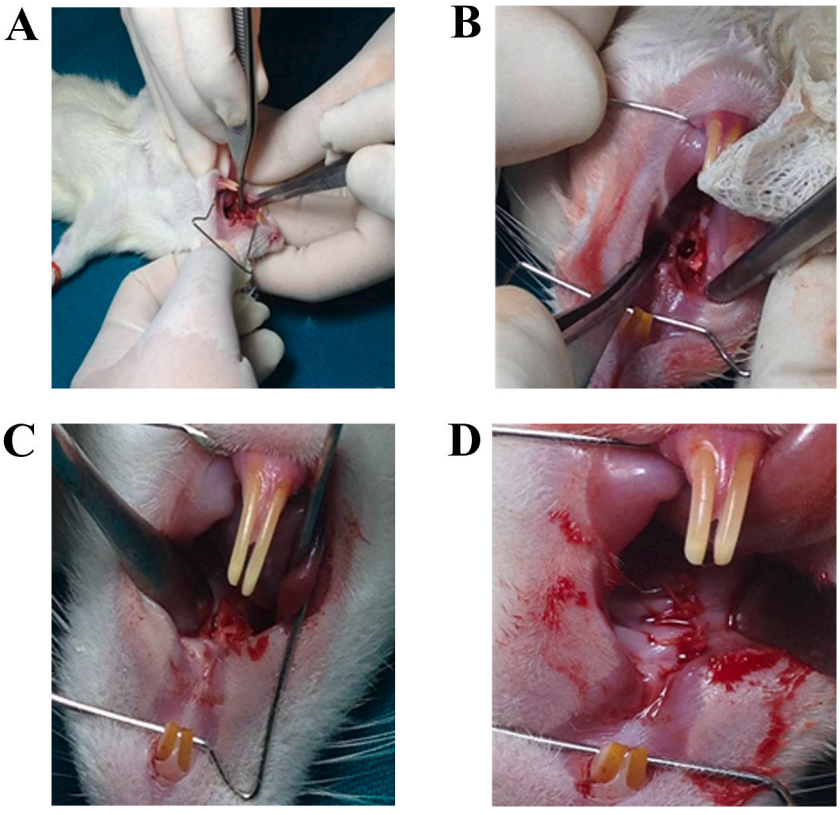

Fig. 1. Establishment of a rat cleft palate model. (A) Surgical blade incision in the right maxillary first molar. (B) Molar extraction and intact alveolar bone drilling. (C) Scaffold transplantation. (D) Surgically sutured gingival tissue.

The animal model comprised five experimental groups (12 rats per group) depending on the type of implanted scaffold. The control group had only the right maxillary first molar extracted and was sutured without implanting any materials. In the second and third groups, rats with a cleft palate-mimicking defect received a PCL-GE scaffold without (PCL-GE) or with SHED (PCL-GE-SHED), respectively. The fourth and fifth groups consisted of rats implanted with a PLGA-BG scaffold without (PLGA-BG) and with SHED (PLGA-BG-SHED).

2.5. Sample collection and gross examination

Rats were daily investigated for their general appearance and appetite. Rats were weighed every week until the end of the experiment. Four rats per group were sacrificed and tissue samples were collected at 2, 3, and 6 months after implantation, respectively. The entire maxilla was dissected, its gross appearance examined, and then fixed in 10 % buffereded formalin. After complete fixation, tissues were decalcified using a decalcifying agent (Decalcifier System, Leica Biosystems In., Buffalo Grove, IL, USA).

2.6. Histological analysis

After decalcification, tissues were routinely processed for histological examination. Four-μm-thick tissue sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin. The numbers of animal which present the bone tissue in the cleft defect were counted. The histological evaluation was examined based on the previous study [22]. Histological findings, including the inflammatory reaction, scaffold availability, and formation of bone tissue, were observed under a light microscope.

2.7. Immunohistochemistry

Four-μm-thick sections on positive charge slides were used for anti-osteocalcin and antihuman mitochondria immunohistochemical studies. For the former, tissue sections were heated in an oven at 60 °C for 45 min, deparaffinized in xylene, and rehydrated before incubation with a citrate-buffered (pH 6.0) antigen retrieval solution in a microwave at a median high temperature for 5 min. Subsequently, tissue sections were washed with phosphate-buffereded saline and blocked with 3 % hydrogen peroxide in methanol for 30 min. Nonspecific binding sites were blocked with 1 % bovine serum albumin. Each section was then washed with phosphate-buffereded saline followed by overnight incubation with anti-osteocalcin antibody ((G-5): sc-365797, Santa Cruz Biotechnology, Inc., Dallas, TX, USA, 1:500 dilution) at 4 °C. Subsequently, the conjugated secondary antibody (Envision™, Dako, Denmark) was added and incubated for 45 min. After washing with phosphate-buffereded saline, chromogen was added before another wash with distilled water. The reaction was visualized with a 3,3-diaminobenzidine substrate and the reaction stopped in distilled water. The tissue sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. For anti-human mitochondrial immunohistochemistry, tissue sections were fixed with 4 % paraformaldehyde in phosphate-buffereded saline for 1 h at room temperature. Then, sections were washed with phosphate-buffereded saline and blocked in 15 % bovine serum albumin. After blocking, sections were permeabilized with 0.5 % Triton X-100 overnight at 4 °C. The primary antibody (human mitochondria, Sigma-Aldrich Corp.,

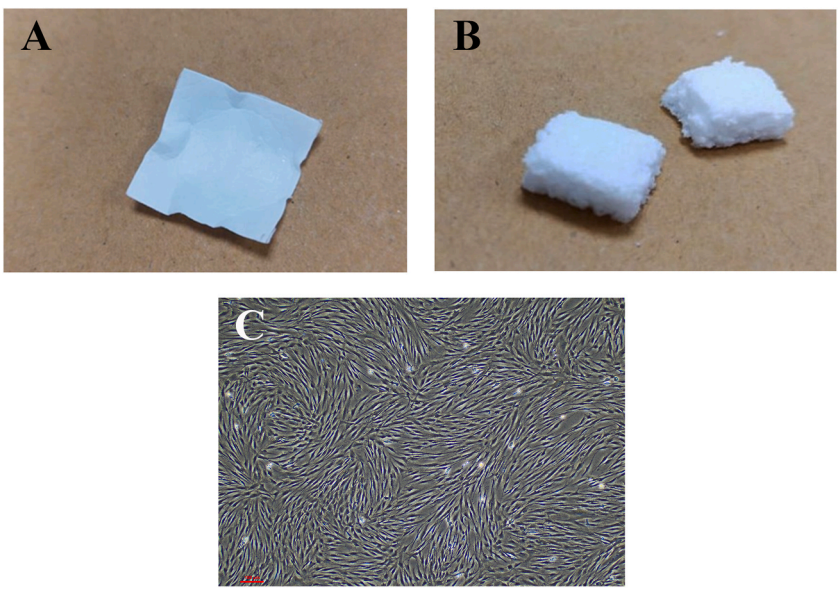

Fig. 2. Morphology of scaffolds and dental stem cells. (A) Electrospun PCL-GE fiber scaffolds. (B) Three-dimensional PLGA with 10 % BG composite scaffolds. (C) Stem cells isolated from deciduous teeth. Scale bar, 100 μm. PCL, polycaprolactone; GE, gelatin; PLGA, poly(lactic-*co*-glycolic acid); BG, bioactive glass.

1:500 dilution) was incubated overnight at 4 °C. The secondary antibody conjugated reagent (Envision™, Dako, DK) was added and incubated for 45 min. Then, chromogen was added to the tissue sections and the reaction visualized with a 3,3-diaminobenzidine (Sigma-Aldrich Corp) substrate; the reaction was stopped with distilled water. The sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. Finally, tissue samples were evaluated for protein expression using light microscopy. Rat bone and human liver tissue were used as positive controls for anti-osteocalcin and anti-human mitochondria, respectively. The positive immunohistochemistry was evaluated according to the previous study [22].

3. Results

3.1. Gross findings

Based on our previous studies [16,17,22], PCL-GE (Fig. 2A) and PLGA with 10 % BG (Fig. 2B) composite scaffolds were selected for transplantation without/with SHED (Fig. 2C) into a rat cleft-mimicking area. After the surgical procedure, all rats had normal appetite and showed no clinical problems throughout the experimental period. There were no significant differences in body weight, blood profiles–including complete blood count–, and blood chemistry between experimental and control groups.

Complete closure of the incised wound at the surgical site was observed in all rats (Fig. 3). No evidence of a new tooth was macroscopically found in any experimental groups. In the cross-sectional sample, replacing the extracted right first molar site revealed small white irregular foci with continuous gingival epithelial lining underlying the defects in all rats (Fig. 3; black arrow head). No hemorrhage or necrotic tissue was observed in either implanted or adjacent areas.

3.2. Histological analysis

The histological results showed complete closure of the gingival epithelium in all groups after two months (Fig. 4A). In several rats, the remaining root tooth was found at the defect site (Fig. 4A). The control group showed little inflammatory reaction (Fig. 4A and B). Fibrous tissue formation (fibrosis) with neovascularization was predominantly found at the defect region over the experimental period in control rats (Fig. 4A). Table 1 shows the number of animals exhibiting bone or osteoid formation. No evidence of osteoid formation was found in any control animals at 2, 3, and 6 months after tooth extraction. In the PCL-GE group, foreign body reaction, including presentation of multinucleated giant cells (osteoclasts), surrounding the fragmented scaffolds and neovascularization, was predominantly observed at 2 and 3 months (Fig. 4B; black arrow). PCL-GE scaffold residues in the tissue were observed as clear elongated or fragmented filaments in the implanted sites at all time points (Fig. 4B; white arrow head). Bone matrix-like structures, or osteoids, were

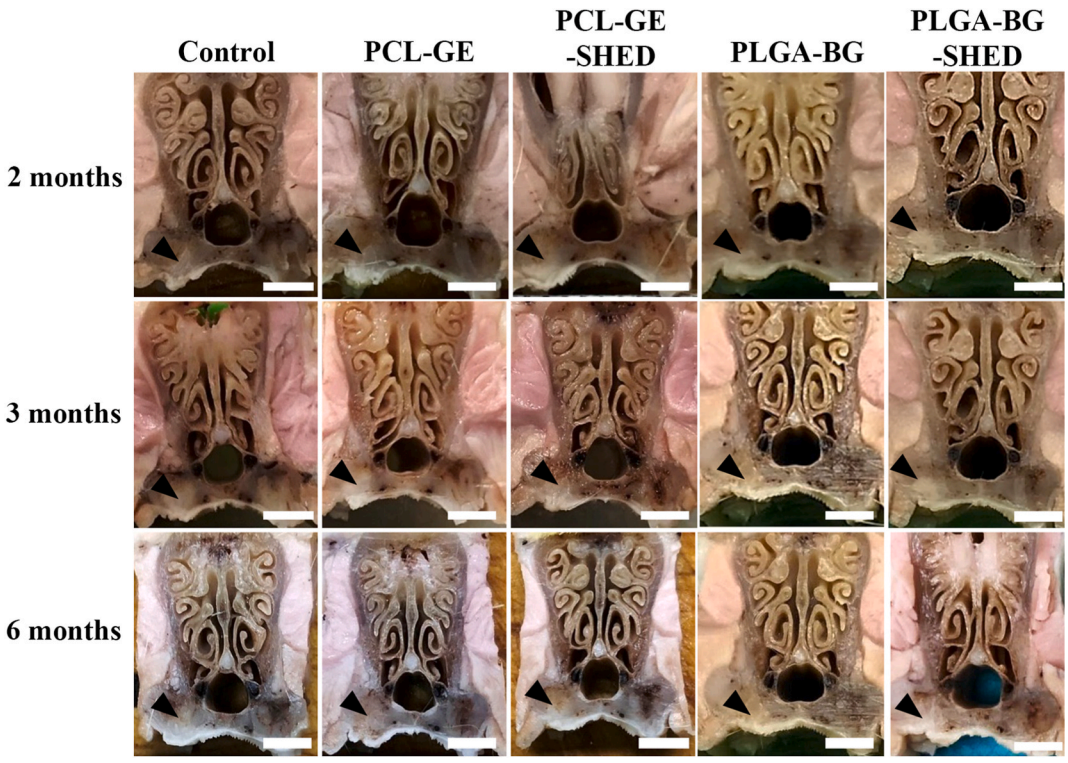

Fig. 3. Maxilla of Sprague-Dawley rats. Complete closure of the incised wound at the surgical site was observed in all rats. Cross-sectional samples reveal small white irregular foci in every rat. Scale bar, 3 mm. Black arrowhead (▲), defect region.

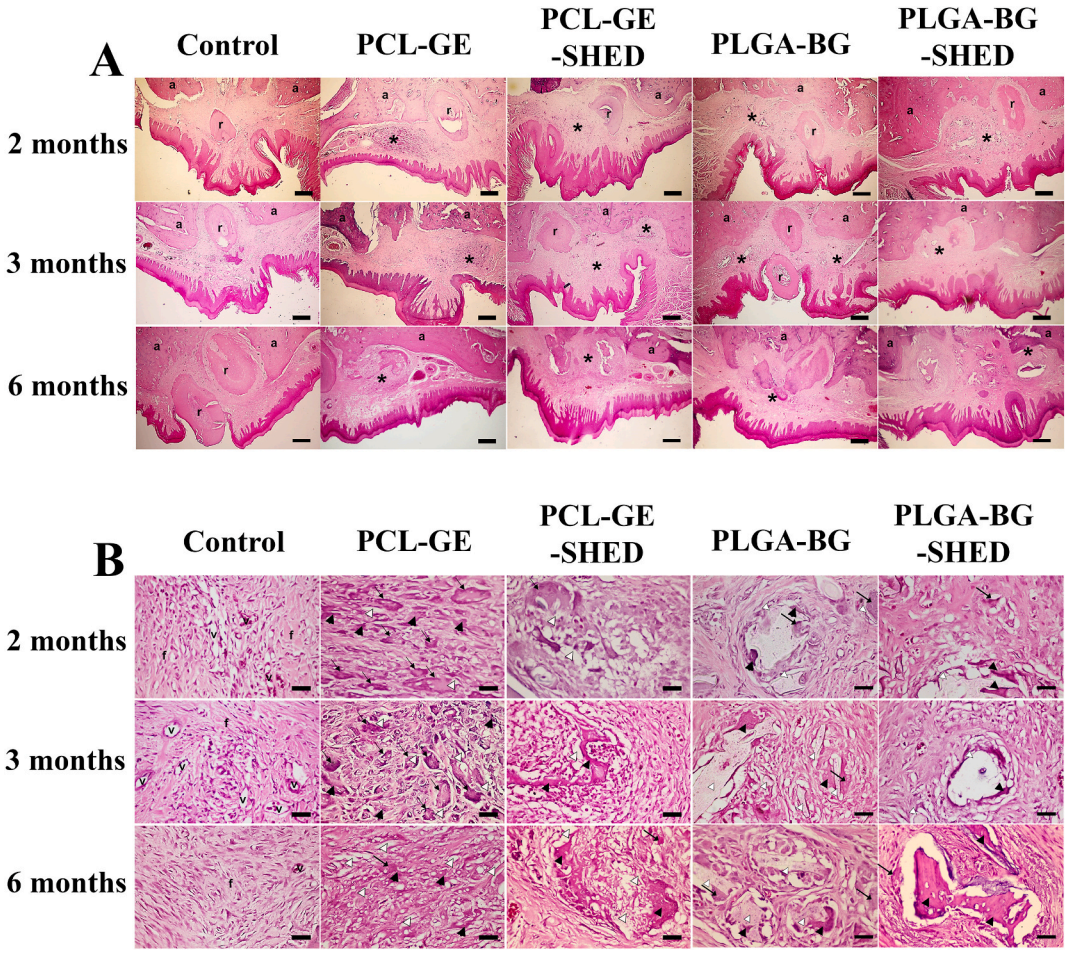

Fig. 4. Histologic findings of osteoid tissue or bone matrix in cleft palate rats after scaffold transplantation. (A) Hematoxylin and eosin staining of control, PCL-GE, PCL-GE-SHED, PLGA-BG, and PLGA-BG-SHED graft areas at 2, 3, and 6 months after transplantation. Scale bar, 300 μm. (B) At high magnification (40x), histological findings of the first molar region from control and scaffold-implanted groups at 2, 3, and 6 months. Scale bar, 300 μm. PCL, polycaprolactone; GE, gelatin; PLGA, poly(lactic-co-glycolic acid); BG, bioactive glass; (*), regions with implanted scaffold; a = alveolar bone; f, fibrosis; r, remaining tooth parts; v, neovascularization; arrow, osteoclast; black arrowhead (▲), bone matrix; white arrowhead (Δ), scaffold residue.

Table 1
Number of rats presenting osteoid or bone tissue formation in the implanted area.

Duration of implantation	Group				
	Control	PCL-GE	PCL-GE -SHED	PLGA-BG	PLGA-BG -SHED
2 months	0/4 (0 %)	1/4 (25 %)	3/4 (75 %)	3/4 (75 %)	4/4 (100 %)
3 months	0/4 (0 %)	2/4 (50 %)	3/4 (75 %)	3/4 (75 %)	4/4 (100 %)
6 months	0/4 (0 %)	3/4 (75 %)	4/4 (100 %)	4/4 (100 %)	4/4 (100 %)

observed in rats at 2 months (1 out of 4 rats), 3 months (2 out of 4 rats), and 6 months (3 out of 4 rats) after implantation, respectively (Table 1). The results of PCL-GE with SHED were similar to those in the PCL-GE group. However, at 2 and 3 months, osteoids were observed in the majority of rats, and this distinctive feature was evident in three out of four rats. At 6 months after implantation of PCL-GE with SHED, all rats displayed these structures (Fig. 4B; black arrow head). PLGA-BG scaffolds appeared as irregular glistening materials in the tissue (Fig. 4B; white arrow head). Similar to PCL-GE, tissue reaction was also observe surrounding the transplanted PLGA-BG scaffold areas. More rats showed regenerated osteoid tissue after PLGA-BG scaffold implantation that after PCL-GE implantation at all time points (Table 1). All rats in the PLGA-BG-SHED group showed osteoid-like structures after transplantation at 2, 3, and 6 months.

3.3. Immunohistochemistry

We observed positive osteocalcin immunoreactivity in all rats exclusively in PCL-GE, PCL-GE-SHED, PLGA–BG, and PLGA-BG-SHED implanted areas (Fig. 5A; white arrows). This indicates the presence of osteoblasts in the transplantation area at 2, 3, and 6 months after transplantation.

To confirm the availability of human dental pulp stem cells in rat tissues, we performed antihuman mitochondrial immunohistochemistry. Control and scaffolds without SHED cells revealed no expression of antihuman mitochondria (Fig. 5B) whereas the PCL-GE-SHED and PLGA-BG-SHED groups exhibited positive immunoreactivity for human mitochondria 2–6 months after implantation (Fig. 5B; black arrows). However, no differences were observed in the intensity of human anti-human mitochondrial immunolabeling at all time points.

4. Discussion

Rodent animal models have been established to study the potency of alternative bone-substitution materials *in vivo* [37–39]. The present study selected rats for creating the cleft palate defect by extraction of the first molar and burring the adjacent alveolar bone tissue and PCL-GE and PLGA-BG scaffolds were used as bone-substitution graft materials. Further, as we had previously found that PCL-GE surface modification and PLGA with 10%BG composite scaffolds can maintain SHED *in vitro* [16,17,22], we studied osteoconductivity and osteoinductivity after implantation in the rat model.

Clinically, no rats suffered or died after scaffold implantation. The rats showed normal appetite, maintained normal weight, and exhibited normal clinical pathology parameters, indicating that both scaffolds with/or without SHEDs were nontoxic. Histologically, all transplanted groups showed a moderate degree of tissue reaction, including infiltration of multinucleated giant cells surrounding the scaffold materials, indicating a biodegradable process as macrophages or multinucleated giant cells are involved in *in vivo* biodegradation of exogenous biomaterials, such as resorbable polymers [40]. However, the current findings indicated that the degradation of PCL-GE and PLGA-BG was not complete at 6 months after implantation, as shown by the remaining material at the implantation sites. In control animals without scaffolds or SHEDs, the vacant cleft regions consistently exhibited filling with fibrous tissue and blood vessels (neovascularization) across all time points. In contrast, the groups implanted with PCL-GE and PLGA-BG demonstrated earlier onset of osteoid formation. Both types of scaffolds exhibit osteoconductive properties, inducing osteogenesis at the defect site.

More regenerated bone tissue was found in rats with PLGA-BG scaffolds than in those with PCL-GE scaffolds throughout the experiments. This distinction may be underpinned by the inherent limitations of PCL, such as its low bioactivity [41] and restricted interaction with biological systems [42]. This indicates that PCL might not ensure essential biological response vital for effective tissue

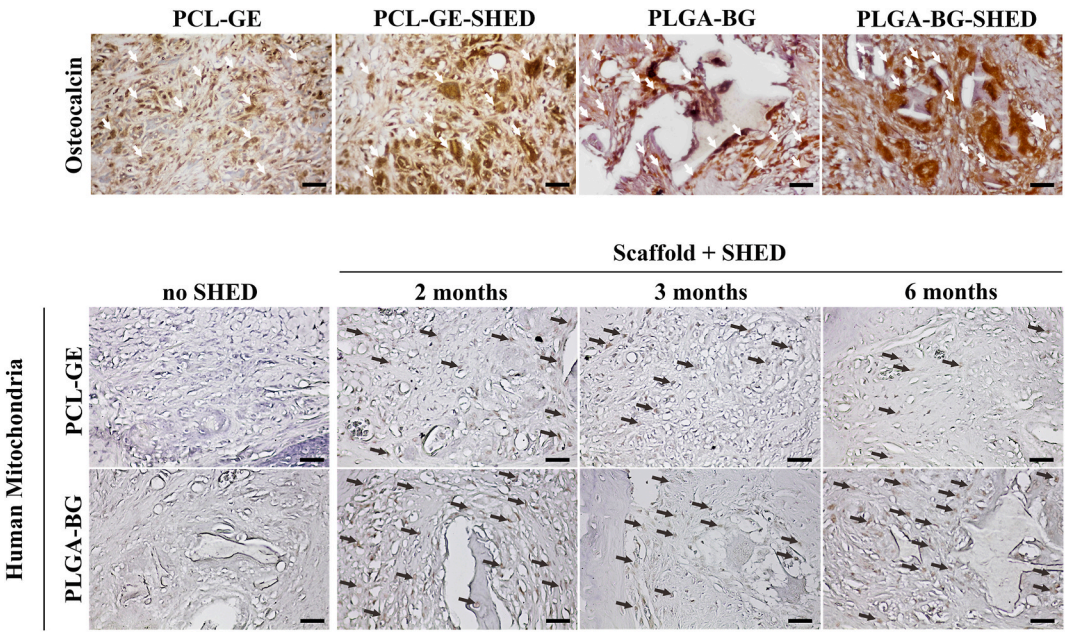

Fig. 5. Immunohistochemistry images of osteoblasts in rats with cleft palate after transplantation. (A) Osteocalcin protein expression in PCL-GE, PCL-GE with SHED, PLGA-BG, and PLGA-BG-SHED (graft area at 6 months after transplantation. (B) Human mitochondria staining of scaffolds with or without SHED graft area at 2, 3, and 6 months after transplantation. A,B: scale bar, 30 μm. PCL, polycaprolactone; GE, gelatin; PLGA, poly (lactic-*co*-glycolic acid); BG, bioactive glass; white arrow, osteocalcin-positive immunolabeling; black arrow, antihuman mitochondria-positive immunolabeling.

regeneration.

Our immunohistochemistry analysis confirmed the histological findings, revealing osteoblasts and osteoid formation in all samples implanted with scaffolds with/or without SHED. The combination of bone substitution materials or scaffolds with precolonized osteoblastic-differentiated stem cells might increase osteoconductivity and osteoinductivity [5]. Notably, after the transplantation of SHED into immunodeficient mice, there is evidence of mineralized tissue formation [31,32]. The current research indicated a tendency for increased bone formation after transplantation of a scaffold with SHED. Moreover, we found positive immunoreactivity in the osteoblast-like cells in the implantation site exclusively surrounding the scaffolds, similar to previous research [22]. Previous studies showed that mesenchymal stem cells, including SHEDs, are alive and proliferate in murine tissue by modulating the host immune response [43]. Proposed cytotoxic inhibitory mechanisms include a reduced secretion of proinflammatory cytokines, such as IFN-γ and TNF-α, which play an important role in inflammation by activating macrophages, cytotoxic T lymphocytes, natural killer cells, and neutrophils [44]. In addition, previous *in vitro* research has shown that SHEDs survive and promote regeneration of trabecular bone after implantation in an overstimulated immune systemic lupus erythematosus (SLE)-like murine model [45]. This report suggested that excellent immunomodulatory effects occurred via suppression of Th17 cells and elevation of regulatory T cells (Tregs). Furthermore, Jahanbin et al. [46] demonstrated that osteoblastic-differentiated SHEDs caused no rejection after implantation in rat tissues. Further studies should more precisely evaluate biodegradation of PCL-GE and PLGA-BG as well as SHED properties in osteogenesis *in vivo* and clinically investigate the application of PCL-GE and PLGA-BG scaffolds with SHED in cleft patients. However, the research findings underscore the need for further investigation due to notable aspects. These include the absence of quantification for bone tissue regeneration across different experimental groups and the level of positivity in immunohistochemistry. There's a compelling interest in future studies to compare osteogenicity levels by evaluating both the volume of bone matrix generation and the intensity of bone-producing proteins within individual samples.

5. Conclusion

In summary, PCL-GE and PLGA-BG scaffolds emerge as potential alternatives for bone-substitution graft materials in the surgical treatment of cleft palate patients. Not only are they biocompatible and nontoxic, but they also induce osteogenesis upon implantation in an animal cleft palate model. While the inclusion of SHEDs in the scaffolds did not exhibit significant positive effects on osteoinductivity, the cells persisted and remained present in the animal tissue throughout the entire experimental period.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Kasem Rattanapinyopituk: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Chaiyapol Chaweewannakorn:** Visualization, Validation, Methodology, Investigation, Formal analysis. **Nathaphon Tangjit:** Visualization, Validation, Methodology, Investigation, Funding acquisition. **Surachai Dechkunakorn:** Visualization, Validation, Investigation, Funding acquisition, Conceptualization. **Niwat Anuwongnukroh:** Visualization, Validation, Investigation, Funding acquisition. **Hathaitip Sritanaudomchai:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] D.J. Crockett, S.L.Goudy SL. Cleft lip, palate Facial, Plast. Surg. Clin. N. Am. 22 (2014) 573–586, https://doi.org/10.1016/j.fsc.2014.07.002.
[2] M.A. Shkoukani, L.A. Lawrence, D.J. Liebertz, P.F. Svider, Cleft palate: a clinical review, Birth. Defects. Res. C. 102 (2014) 333–342, https://doi.org/10.1002/bdrc.21083.
[3] A.M. Dao, S.L. Goudy, Cleft palate repair, gingivoperiosteoplasty, and alveolar bone grafting, Facial. Plast. Surg. Clin. N. Am. 24 (2016) 467–476, https://doi.org/10.1016/j.fsc.2016.06.005.

[4] S.E. Watkins, R.E. Meyer, R.P. Strauss, A.S. Aylsworth, Classification, epidemiology, and genetics of orofacial clefts, Clin. Plast. Surg. 41 (2014) 149–163, https://doi.org/10.1016/j.cps.2013.12.003.

[5] P. Korn, T. Ahlfeld, F. Lahmeyer, D. Kilian, P. Sembdner, R. Stelzer, W. Pradel, A. Frankie, M. Rainer, U. Range, B. Stadlinger, A. Lode, G. Lauer, M. Gelinsy, 3D printing of bone grafts for cleft alveolar osteoplasty - *In vivo* evaluation in a preclinical model, Front. Bioeng. Biotechnol. 8 (2020) 217, https://doi.org/10.3389/fbioe.2020.00217.

[6] M. Martín-del-Campo, R. Rosales-Ibañez, L. Royo, Biomaterials for cleft lip and palate regeneration, Int. J. Mol. Sci. 20 (2019) 2176, https://doi.org/10.3390/ijms20092176.

[7] D.M. Fisher, B.C. Sommerlad, Cleft lip, cleft palate, and velopharyngeal insufficiency, Plast. Reconstr. Surg. 128 (2011) 342e–360e, https://doi.org/10.1097/PRS.0b013e3182268e1b.

[8] R. Jayarum, C. Huppa, Surgical correction of the cleft lip and palate, Front. Oral. Bill. 16 (2012) 101–110, https://doi.org/10.1159/000337664.

[9] M. Berger, F. Proust, C. Schwartz, M. Cornelsen, H. Seitz, M. Ehrenfeld, S. Otto S, A concept for scaffold-based tissue engineering in alveolar cleft osteoplasty, J. Cranio-Maxillo-Fac. Surg. 43 (2015) 830–836, https://doi.org/10.1016/j.jcms.2015.04.023.

[10] E.H. Weissler, K.M. Paine, M.K. Ahmed, P.J. Taub, Alveolar bone grafting and cleft lip and palate: a review, Plast. Reconstr. Surg. 138 (2016) 1287–1295, https://doi.org/10.1097/prs.0000000000002778.

[11] R. Izquierdo, N. Garcia-Gibraltar, M.T. Rodriguez, E. Cáceres, S.J. García, J.L.G. Ribelles, M. Monleón, J.C. Monllau, J. Suay, Biodegradable PCL scaffolds with an interconnected spherical pore network for tissue engineering, J. Biomed. Mater. Res. A. 85 (2007) 25–35, https://doi.org/10.1002/jbm.a.31396.

[12] P. Gentile, V. Chiono, I. Carmagnola, P.V. Hatton, An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering, Int. J. Mol. Sci. 15 (2014) 3640–3659, https://doi.org/10.3390/ijms15033640.

[13] N. Siddiqui, S. Asawa, B. Biarritz, R. Baadhe, S. Rayo, PCL-based composite scaffold matrices for tissue engineering applications, Mol. Biotechnol. 60 (2018) 505–532, https://doi.org/10.1007/s12033-018-0084-5.

[14] T. Patrício, M. Domingos, A. Gloria, P. Bártolo, Characterisation of PCL and PCL/PLA scaffolds for tissue engineering. Procedia, CIRP. 5 (2013) 110–114, https://doi.org/10.1016/j.procir.2013.01.022.

[15] R. Dwivedi, S. Kumar, R. Pandey, R. Mahajan, D. Nandana, D.S. Kati, D. Mehrotra, Polycaprolactone as biomaterial for bone scaffolds: review literature, J. Oral. Biol. Craniofac Res. 10 (2020) 381–388, https://doi.org/10.1016/j.jobcr.2019.10.003.

[16] J. Jitpibull, T. Vongsetskul, H. Sritanaudomchai, N. Tangjit, Surface-functionalized electrospun polycaprolactone fiber for culturing stem cell from human exfoliated deciduous teeth culture, Fibers Polym. 21 (2020) 2215–2223, https://doi.org/10.1007/s12221-020-1147-3.

[17] J. Jitpibull, N. Tangjit, S. Dechkunakorn, N. Anuwongnukroh, T. Srikhirin, T. Vongsetskul, H. Sritanaudomchai, Effect of surface chemistry modified polycaprolactone scaffolds on osteogenic differentiation of stem cells from human exfoliated deciduous teeth, Eur. J. Oral Sci. 129 (2021) e12766, https://doi.org/10.1111/eos.12766.

[18] S. Shaneh, F. Shokrolahi, P. Shokrollahi, H. Yeganeh, E. Seyedjafari, A. Ardeshirylajimi, H. Omidian, Improved immobilization of gelatin on a modified polyurethane urea, J. Bioact. Compat Polym. 30 (2014) 57–73, https://doi.org/10.1177/0883911518819224.

[19] V. Guarino, M. Alvarez-Perez, V. Cirillo, L. Ambrosio, hMSC interaction with PCL and PCL/gelatin platforms: a comparative study on films and electrospun membranes, J. Bioact. Compat Polym. 26 (2011) 144–160, https://doi.org/10.1177/0883911511399410.

[20] Z. Pan, J. Ding, Poly(lactide-co-glycolide) porous scaffolds for tissue engineering and regenerative medicine. Interface, Focus 2 (2012) 366–377, https://doi.org/10.1098/rsfs.2011.0123.

[21] N.T. Hiep, B. Lee, Electro-spinning of PLGA/PCL blends for tissue engineering and their biocompatibility, J. Mater. Sci. Mater. Med. 21 (2010) 1969–1978, https://doi.org/10.1007/s10856-010-4048-y.

[22] N. Kunwong, N. Tangjit, K. Rattanapinyopituk, S. Dechkunakorn, N. Anuwongnukroh, T. Arayapisit, H. Sritanaudomchai, Optimization of poly (lactic-co-glycolic acid)-bioactive glass composite scaffold for bone tissue engineering using stem cells from human exfoliated deciduous teeth, Arch. Oral Biol. 123 (2021) 105041, https://doi.org/10.1016/j.archoralbio.2021.105041.

[23] J.K. Leach, D. Kaigler, Z. Wang, P.H. Krebsbach, D.J. Mooney, Coating of VEGF-releasing scaffolds with bioactive glass for angiogenesis and bone regeneration, Biomaterials 27 (2006) 3249–3255, https://doi.org/10.1016/j.biomaterials.2006.01.033.

[24] J. Filipowska, J. Pawlik, K. Cholewa-Konwalska, G. Tylko, E. Pamula, L. Niedzwiedzki, M. Szuta, M. Laczka, A.M. Osyczka, Incorporation of sol-gel bioactive glass into PLGA improves mechanical properties and bioactivity of composite scaffold and results in their osteoinductive properties, J. Biomed. Mater. 9 (2014) 1–10, https://doi.org/10.1088/1748-6041/9/6/065001.

[25] E. Tavakoli, M. Mehdikhani-Nahrkhalaji, B. Hashemi-Beni, A. Zargar-Kharazi, Kharaziha. Preparation, characterization and mechanical assessment of poly (lactide-co-glycolide)/hyaluronic acid/fibrin/bioactive glass nano-composite scaffold for cartilage tissue engineering applications, Procedia. Mater. Sci. 11 (2015) 124–130, https://doi.org/10.1016/j.mspro.2015.11.126.

[26] N. Cui, J. Qian, J. Wang, C. Ji, W. Xu, H. Wang, Preparation physicochemical properties and biocompatibility of PBLG/PLGA/bioglass composite scaffold, Mater. Sci. Eng. 27 (2017) 3249–3255, https://doi.org/10.1016/j.msec.2016.09.085.

[27] S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi, Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 13625–13630, https://doi.org/10.1073/pnas.240309797.

[28] M. Miura, S. Gronthos, M. Zhao, B. Lu, L.W. Fisher, P.G. Robey, S. Shi, SHED: stem cells from human exfoliated deciduous teeth, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 5807–5812, https://doi.org/10.1073/pnas.0937635100.

[29] Y. Nishino, Y. Yamada, K. Ebisawa, S. Nakamura, K. Okabe, E. Umemura, K. Hara, M. Ueda, Stem cells from human exfoliated deciduous teeth (SHED) enhance wound healing and the possibility of novel cell therapy, Cytotherapy 13 (2011) 598–605, https://doi.org/10.3109/14653249.2010.542462.

[30] N. Zhang, B. Chen, W. Wang, C. Chen, J. Kang, S.Q. Deng, B. Zhang, S. Liu, F. Han, Isolation, characterization and multi-lineage differentiation of stem cells from human exfoliated deciduous teeth, Mol. Med. Rep. 14 (2016) 95–102, https://doi.org/10.3892/mmr.2016.5214.

[31] X. Wei, J. Ling, L. Wu, L. Liu, Y. Xiao, Expression of mineralization markers in dental pulp cells, J. Endod. 33 (2007) 703–708, https://doi.org/10.1016/j.joen.2007.02.009.

[32] M. Kitagawa, H. Ueda, S. Iizuka, K. Sakamoto, H. Oka, Y. Kudo, I. Ogawa, M. Miyauchi, H. Tahara, T. Takata, Immortalization and characterization of human dental pulp cells with odontoblastic differentiation, Arch. Oral Biol. 52 (2007) 727–731, https://doi.org/10.1016/j.archoralbio.2007.02.006.

[33] S. Gautam, A.K. Dinda, A.C. Mishra, Fabrication and characterization of PCL/gelatin composite nanofibrous scaffold for tissue engineering applications by electrospinning method, J. Mater. Sci. Mater. Med. 33 (2013) 1228–1235, https://doi.org/10.1016/j.msec.2012.12.015.

[34] H. Pirayesh, J.A. Nychka, Sol–Gel synthesis of bioactive glass-ceramic 45S5 and its *in vitro* dissolution and mineralization behavior, J. Am. Ceram. Soc. 96 (2013) 1643–1650, https://doi.org/10.1111/jace.12190.

[35] T. Gonmanee, C. Thonabulsombat, K. Vongsavan, H. Sritanaudomchai, Differentiation of stem cells from human deciduous and permanent teeth into spiral ganglion neuron-like cells, Arch. Oral Biol. 88 (2018) 34–41, https://doi.org/10.1016/j.archoralbio.2018.01.011.

[36] N. Tangjit, S. Dechkunakorn, N. Anuwongnukroh, A. Khaneungthong, H. Sritanaudomchai, Optimal xeno-free culture condition for clinical grade stem cells from human exfoliated deciduous teeth, Int. J. Stem. Cells. 11 (2018) 96–104, https://doi.org/10.15283/ijsc17049.

[37] D.Y.S. Tanikawa, C.C.G. Pinheiro, M.C.A. Almeida, C.R.G.C.M. Oliveira, R.A.C. Coudry, D.L. Rocha, D.F. Bueno Df, Deciduous dental pulp stem cells for maxillary alveolar reconstruction in cleft lip and palate patients, Stem Cell. Int. 1 (2020) 1–9, https://doi.org/10.1155/2020/6234167.

[38] O.O. Aalami, R.P. Nacamuli, K.A. Lenton, C.M. Cowan, T.D. Fang, K.D. Fong, Y. Shi, H.M. Song, D.E. Sahar, M.T. Longaker, Applications of a mouse model of calvarial healing: differences in regenerative abilities of juveniles and adults, Plast. Reconstr. Surg. 114 (2004) 713–720, https://doi.org/10.1097/01.prs.0000131016.12754.30.

[39] P.D. Nguyen, C.D. Lin, A.C. Allori, J.L. Ricci, P.B. Saadeh, S.M. Warren, Establishment of a critical-sized alveolar defect in the rat: a model for human gingivoperiosteoplasty, Plast. Reconstr. Surg. 123 (2009) 817–825, https://doi.org/10.1097/PRS.0b013e31819ba2f4.

[40] Z. Sheikh, P.J. Brooks, O. Barzilay, N. Fine, M. Glogauer, Macrophages, foreign body giant cells and their response to implantable biomaterials, Materials 8 (2015) 5671–5701, https://doi.org/10.3390/ma8095269.

[41] J.K. Hong, S.L. Cooke, A.R. Whittington, M. Roman, Bioactive cellulose nanocrystal-poly(ε-caprolactone) nanocomposites for bone tissue engineering applications, Front. Bioeng. Biotechnol. 9 (17) (2021) 632029, 9, https://doi:10.3389/fbioe.2021.632029.
[42] S. Zhao, K. Xie, Y. Guo, J. Tan, J. Wu, Y. Yang, P. Fu, L. Wang, W. Jiang, Y. Hao, Fabrication and biological activity of 3D-printed polycaprolactone/magnesium porous scaffolds for critical size bone defect repair, ACS Biomater. Sci. Eng. 6 (2020) 5120–5131. https://doi:10.1021/acsbiomaterials.9b01911.
[43] R.D. Chapurlat, C.B. Confavreux, Novel biological markers of bone: from bone metabolism to bone physiology, Rheumatology 55 (2016) 1714–1725, https://doi.org/10.1093/rheumatology/kev410.
[44] L. Wang, Y. Zhao, S. Shi, Interplay between mesenchymal stem cells and lymphocytes: implications for immunotherapy and tissue regeneration, J. Dent. Res. 91 (2012) 1003–1010, https://doi.org/10.1177/0022034512460404.
[45] T. Yamaza, A. Kentaro, C. Chen, Y. Liu, Y. Shi, S. Gronthos, S. Wang, Immunomodulatory properties of stem cells form human exfoliated deciduous teeth, Stem Cell Res. Ther. 1 (2010) 5, https://doi.org/10.1186/scrt5.
[46] A. Jahanbin, R. Rashed, D.H. Alamdari, N. Koohestanian, A. Ezzati, M. Kazemin, S. Sagdi, M.A. Raisolsadat, Success of Maxillary alveolar defect repair in rats using osteoblast-differentiated human deciduous dental pulp stem cells, J. Oral Maxillofac. Surg. 74 (2016) 829.e1–829.e9, https://doi.org/10.1016/j.joms.2015.11.033.