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Human periodontal ligament cells reaction on a novel hydroxyapatite–collagen scaffold

Jing Guo[1,2], Ying Wang[1], Chengbo Cao[3], Rosemary Dziak[4], Brian Preston[1], Guoqiang Guan[1]

[1]Department of Orthodontics, School of Dental Medicine, University at Buffalo, State University of New York, Buffalo, NY, USA; [2]Department of Orthodontics, School & Hospital of Stomatology, Shandong University; [3]School of Chemistry and Chemical Engineering, Shandong University, Jinan, Shandong, China; [4]Department of Oral Biology, School of Dental Medicine, University at Buffalo, State University of New York, Buffalo, NY, USA

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Correspondence to: Guoqiang Guan, Department of Orthodontics, School of Dental Medicine, University at Buffalo, State University of New York, 3435 Main street, 140 Squire Hall, Buffalo, NY 14214, USA
Tel.: +1 716 829 6190
Fax: +1 716 829 2572
e-mail: gguan@buffalo.edu

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Jing Guo and Ying Wang contributed equally to this work.

Abstract – *Background*: Periodontal tissue regeneration presents a highly promising method for restoring periodontal structures. The development of a suitable bioactive scaffold that promotes cell proliferation and differentiation is critical in periodontal tissue engineering. The aim of this study was to evaluate the biocompatibility of a novel 3-dimensional hydroxyapatite–collagen scaffold with human periodontal ligament (hPDL) cell culture. *Methods*: The scaffold was produced from a natural collagen matrix - purified porcine acellular dermal matrix (PADM), which was then treated with hydroxyapatite (HA) through a biomimetic chemical process to obtain hydroxyapatite–porcine acellular dermal matrix (HA-PADM) scaffold. The hPDL cells were cultured with HA-PADM scaffolds for 1, 3, 6, 14, and 28 days. The cell viability assay, scanning electron microscopy (SEM), hematoxylin and eosin (H&E) staining, immunohistochemistry, and confocal microscopy were employed in different time points to evaluate the biocompatibility of the scaffolds with hPDL cells. *Results*: The cell viability assay (WST-1 test) verified cell proliferation on the HA-PADM scaffolds. The SEM study showed unique morphology of hPDL cells, which attach and spread on the surface of the scaffolds. The H&E staining, immunohistochemistry, and confocal microscopy demonstrated that hPDL cells were able to grow into the HA-PADM scaffolds and maintain viability after prolonged culture. *Conclusions*: This study proved that HA-PADM scaffold is - biocompatible for hPDL cells. The cells were able to proliferate and migrate into the scaffold. These observations suggest that HA-PADM is a potential cell carrier for periodontal tissue regeneration.

Damage to the periodontal structures may result from accidental trauma or from periodontal diseases. In both of these situations, the induced inflammatory changes in the periodontal structures usually cause periodontitis, which is an inflammatory disease that invades the periodontium including the gingivae, periodontal ligament, cementum, and alveolar bone. This pathologic condition affects approximately 50% of adults, with as much as 10–30% of these patients displaying bone resorption and ultimately tooth loss (1, 2). While current periodontal treatments generally succeed in removing the pathogenic bacteria and in arresting the progress of the periodontal disease destruction, it is still a challenge for dental professionals to restore the structure and function of the damaged periodontium. With recent advances in stem cell research and bioengineering technology, periodontal tissue regeneration has become the most promising method for restoring periodontal structures (3, 4). With periodontal tissue regeneration, it is necessary to obtain a biodegradable scaffold for the delivery of cells to the wound site, as well as for space preservation for the formation of the new periodontal tissue (5, 6). The biologic rationale for using a scaffold to facilitate the regeneration of the periodontal tissues is based on the expectation that the scaffold will support the growing cells of the periodontium while also encouraging alveolar bone formation. The ideal scaffold for periodontal tissue engineering should be biodegradable and relatively easy to handle. These desirable features will encourage cell incorporation and also the free diffusion of the bone-inducing substances that serve as a framework for bone formation. Concerns regarding the current available scaffolds include unforeseeable cell–biomaterial interactions, uneven degradation of the biomaterial, inflammatory reactions, and limited cell seeding efficiency (3, 4). Recently, porcine acellular dermal matrix (PADM) has been developed as a dermal substitute that has been used successfully in skin grafts (7). PADM is mainly composed of type I and type II collagen with a natural 3-dimensional network struc-

ture. It has been shown that after the removal of the cells and cellular components of the porcine skin, PADM has excellent biocompatibility and mechanical properties (8). In this study, we proposed to modify purified PADM with hydroxyapatite (HA) to build an osteoconductive scaffold for periodontal tissue regeneration. The HA–porcine acellular dermal matrix (HA-PADM) scaffold is a two-level pore structure, with large channels (approximately 100 μm in diameter) from the purified PADM microstructure and small pores (<100 nm in diameter) formed by self-assembled HA on the channel surfaces (9). This study was designed to evaluate the *in vitro* biocompatibility of the HA-PADM with a human periodontal ligament (hPDL) cell culture system.

Materials and methods

Preparation of porcine skin scaffolds

Porcine acellular dermal matrix scaffold

Fresh porcine skin was purchased from a local slaughter house. The PADM scaffold was prepared as described patent (CN03139063.3). Briefly, after complete cleaning, excision of the subdermal fat tissue, and removal of hair, the skin was cut into 1.0-mm-thick pieces and purified through basic processing and with enzymatic extraction methods designed to remove the fat and cellular components. The final product was washed carefully with distilled water, and the moisture-laden purified porcine skin was frozen at −80°C. After lyophilization at −60°C for 4 h, a PADM framework was obtained.

Construction of HA-PADM scaffold

The PADM framework was first cut into 10 × 10 × 1 mm sample squares. Twenty of the PADM squares were precalcified through rinsing in 400 ml of 0.1 M $CaCl_2$, K_2HPO_4, and $CaCl_2$ solution for 24 h in an incubator shaker (120 rpm, 37°C) using an alternative soaking method. Double-deionized H_2O washing for 20 min at a time was performed at every two steps to remove the free and weakly connected Ca^{2+} and HPO_4^{2-}. Finally, the precalcified PADM framework was mineralized in one liter simulated body fluid in an incubation box at 37°C for 30 days to obtain HA-PADM scaffolds. The HA-PADM scaffolds were then washed completely and lyophilized at -60°C for 4 h in a vacuum lyophilizer.

Sterilization of the materials

The constructed HA-PADM samples were then cut into small pieces (5 × 5 × 1 mm) and sterilized with ethylene oxide (EO) gas for further use.

Human periodontal ligament cells and cultures

The hPDL cells were isolated from human extracted teeth as described previously (10). The cells were maintained in a minimal essential medium (αMEM) with 10% fetal bovine serum (FBS) and antibiotics and kept in a humidified incubator at 37°C in a 5% CO_2 atmosphere. The characteristics of hPDL cells were confirmed with an immunohistochemical protocol described in the section of histology and immunohistochemistry.

Culture hPDL cells with the scaffolds

The hPDL cells were used at passage two to four in the experiments. After 90% confluence, cells were digested by 0.25% trypsin for 2 min and then neutralized with αMEM medium (10% FBS). Cell density was adjusted to 1×10^5 cells ml^{-1}. The small-sized scaffold samples (5 × 5 × 1 mm) were first placed into a 48-well plastic culture plate, one piece per well. Subsequently, 0.2 ml of the hPDL cell suspension was dropped onto each of the dried scaffolds. After the scaffolds were soaked, an additional 0.3 ml αMEM medium (10% FBS) was added to makeup a total of 0.5 ml of medium for each well. The cells were kept in a humidified incubator at 37°C in 5% CO_2 atmosphere, and the medium was changed every 3 days. The samples ($n = 6$) were collected at day 1, 3, 6, 14, or 28 for different experimental needs. Gelfoam® (5 × 5 × 1 mm) frameworks (Pharmacia & Upjohn Co, New York, NY, USA) were prepared and used as the control scaffold ($n = 6$).

The cell viability/toxicity assay

To evaluate the cytotoxicity of the system, the hPDL cells were cultured with the scaffolds for 1, 3, and 6 days. The cell proliferation reagent WST-1 (Roche Applied Science, Indianapolis, IN, USA) was used to detect the metabolic activity of the cells. At each time point, one tenth of WST-1 reagent was added to the culture medium present in the wells. After the cells were incubated with the reagent for 3 h in a 37°C, 5% CO_2 environment, 0.1 ml of developed media/reagent from each well was transferred to a new 96-well plate and its absorbance was measured at 450 nm on a microplate reader AD340 (Beckman Coulter Inc., Brea, CA, USA).

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the morphological characteristics of the hPDL cells cultured with the scaffold. The hPDL cells were cultured on the scaffolds for 1, 3, and 6 days at 37°C in a CO_2 incubator. At each time point, the scaffolds with the cells were washed twice with phosphate-buffered saline (PBS). The scaffolds with the cells were then fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer for 1 h at room temperature. After being rinsed three times for 15 min with 0.1 M sodium cacodylate buffer, the samples were dehydrated with graded series of ethanol solutions (35%, 50%, 70%, 80%, 95%, and 100%) each for 20 min, and then finally hexamethyldisazane was added for to the samples for 10 min. The samples were then air-dried and mounted on stubs, carbon-coated with a sputter coater, and examined by scanning electron microscope (Hitachi SU70; Hitachi Instruments, Schaumburg, IL, USA).

Histology and immunohistochemsitry

For histological study, the cells cultured with the scaffolds for 1, 3, 6, 14, and 28 days at 37°C in a CO_2 incubator were fixed with 4% formaldehyde in PBS for 24 h before being processed to paraffin embedding. The sections with 5 μm thickness were stained with hematoxylin and eosin (H&E), and then images were taken with a light microscope.

The specimens used for the immunohistochemical study were taken at the same time points as were those used for the histological study. Frozen sections of 7 μm thickness obtained from the scaffolds, and cells cultured on slides were fixed with 4% formaldehyde in PBS for 30 min. The sections were blocked with 10% normal goat serum in PBS for 2 h at room temperature. Primary antibodies used for the staining included mouse monoclonal antibodies for collagen III (Chemicon, Temecula, CA, USA) and Tubulin-α (Invitrogen, Carlsbad, CA, USA) and the secondary antibody was Alexa Fluor® 546 anti-mouse IgG (Invitrogen). Alexa Fluor® 488 Phalloidin and DAPI (Invitrogen) was used to stain filamentous actin (F-actin) and nuclei, respectively. Slides were observed by using a fluorescence microscope (Zeiss AX10; Carl Zeiss, Thornwood, NY, USA) or a confocal microscope LS510 Meta NLO (Carl Zeiss AG, Jena, Germany).

Statistical analysis

The results were expressed as the mean values with standard deviations. Significance was determined using the Student's *t*-test. $P < 0.01$ was considered to be significant.

Results

Characterization of hPDL cells

Immunohistochemical staining of the hPDL cells on slides indicated that these cells expressed collagen III (Fig. 1). In addition, these cells displayed a spindle or spindle-like morphology. Collagen III is a periodontal ligament cell marker (10). The positive staining of Collagen III confirmed that these cells maintained the characteristics of PDL cells after 2–4 passages.

Cytocompatibility analysis of the scaffolds

Figure 2 illustrates the results obtained with the WST-1 assay of the hPDL cells cultured with scaffolds for 1, 3, and 6 days. The optical density (OD) values increased with time in the cell control, Gelfoam, and HA-PADM groups. These results indicated that hPDL cells were able to proliferate on HA-PADM. The OD values in the Gelfoam group at day 6 were the highest and this difference was significant as compared with the HA-PADM group, suggesting more cell growth takes place in the presence of Gelfoam. To understand this finding, we performed further studies to investigate the cell morphology on the HA-PADM and Gelfoam scaffolds.

Demonstration of the hPDL cell attachment and migration

In general, cell attachment, spreading, and proliferation on the scaffold reflect the ability of the scaffold to make contact with the cells. SEM images showed hPDL cells attached to the HA-PADM and Gelfoam scaffolds from day 1 (Fig. 3). However, the cell morphologies on the two scaffolds were totally different. The HA-PADM scaffold had a relatively dense microstructure and the cells attached to the HA-PADM scaffold exhibited a star-shaped appearance with multiprocesses. The cells were well distributed and well attached on the surface of the HA-PADM scaffold. We also observed that the cells communicated with each other by their processes. After incubation for 6 days, cells proliferated extensively on the surface of HA-PADM scaffold. On the other hand, under the conditions of this experiment, Gelfoam provided a more porous three-dimensional network with highly smooth curly sheets that eliminated the pressures that may be imposed in a normal physical environment. The hPDL cells were characterized with an extremely flattened and stretched shape, which attached firmly on the sheets in Gelfoam. The connections between cells were not as well defined as was the case with the cells on the HA-PADM scaffold.

Hematoxylin and eosin staining as well as immunohistochemistry staining provided the cross-sectional views of the scaffolds cultured with the cells. We observed hPDL cells grown on the surface and inside of the HA-PADM and Gelfoam scaffolds (Fig. 4). After prolonged culture, the hPDL cells were able to migrate into the HA-PADM scaffolds at certain spots.

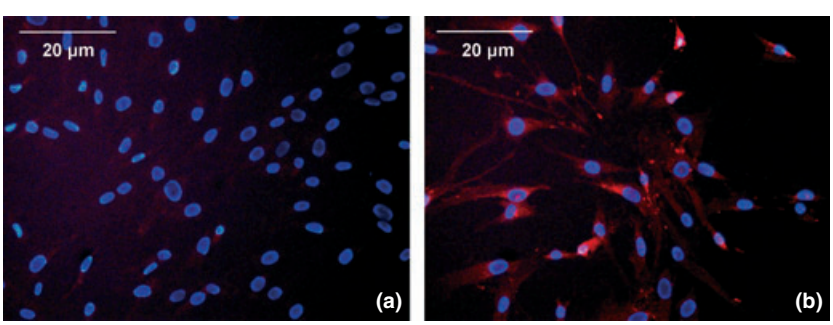

Fig. 1. Collagen III staining of human periodontal ligament (hPDL) cells. hPDL cells were treated with phosphate-buffered saline (a) or anti-Collagen III antibody (b), followed by Alexa Fluor® 546 anti-mouse IgG as secondary antibody (red). Cell nuclei were stained with DAPI in light blue.

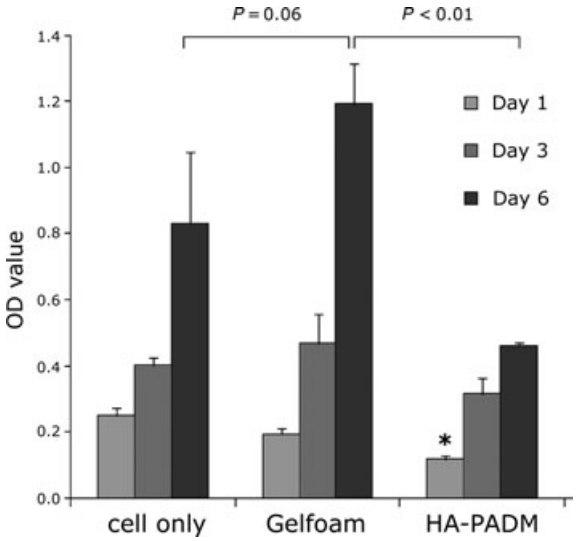

Fig. 2. Cytotoxicity of the scaffolds. Cellular metabolic activity was measured as a means of detecting cell proliferation at day 1, day 3, and day 6. All the cells remained viable and proliferative for control (cell only), Gelfoam, and HA-PADM groups. Symbol (*) represents a statistically significant difference as compared with the optical density (OD) value of cell only group ($P < 0.01$). No statistically significant difference was presented between the OD value of day 6 for cell only and Gelfoam ($P = 0.06$); but a statistically significant difference was presented between the OD value of day 6 for Gelfoam and HA-PADM ($P < 0.01$).

At day 14 and day 28, we observed cells that proliferated and migrated into the deep area of the HA-PADM scaffolds. The sections of Gelfoam showed hPDL cells lined up on the porous structure. After 4 weeks of culture, the cells filled up the spaces in the Gelfoam scaffold. These observations were consistent with our WST-1 assay and SEM findings that Gelfoam provides more room for cells to grow.

Another interesting finding from the immunohistochemistry staining is that the F-actin expression level was much higher in the hPDL cells on HA-PADM specimens as compared to the cells on Gelfoam, indicating that the hydroxyapatite and collagen components of the HA-PADM scaffold promoted hPDL cellular differentiation.

In addition, the confocal microscopy imaging at day 6 (Fig. 5) further verified that the hPDL cells had grown inside the HA-PADM scaffold and spread along the direction of the collagen fibers.

Discussion

Using collagen as a biomaterial for scaffold in tissue engineering has its disadvantages as compared to some other materials. These disadvantages include its low biomechanical stiffness and rapid biodegradation. Further, the high rate of enzymatic degradation of natural collagen *in vivo* makes stabilization of collagen-based biomaterials necessary (11, 12). Moreover, for tissue engineering, previous studies have demonstrated that

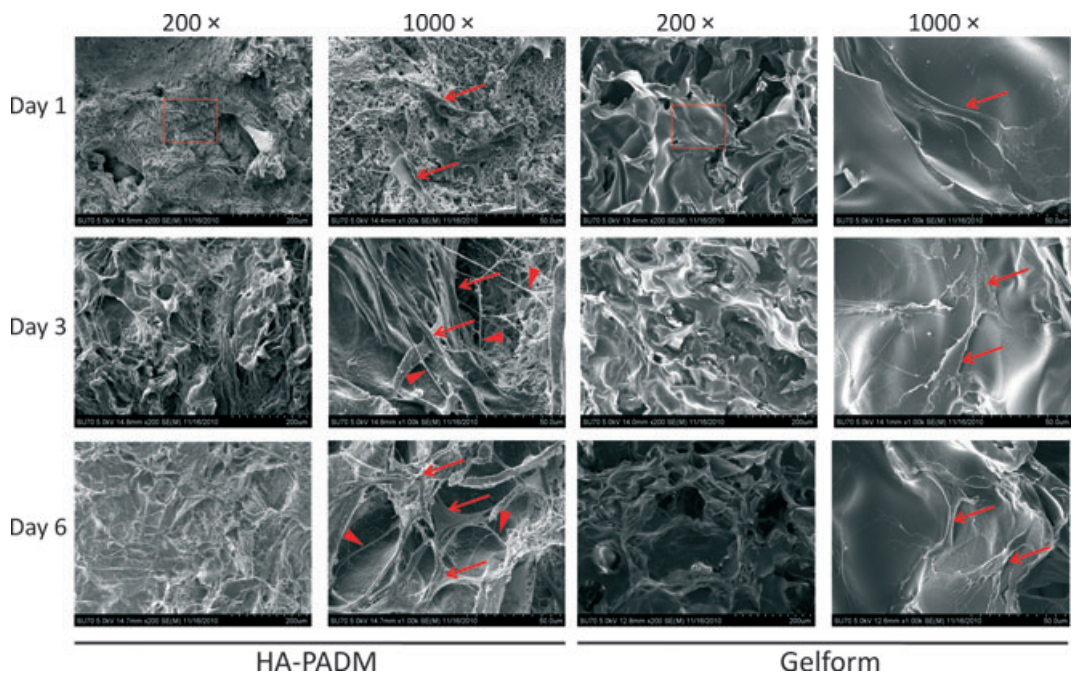

Fig. 3. Scanning electron microscopy imagining showed the morphology of human periodontal ligament (hPDL) cells on hydroxyapatite–porcine acellular dermal matrix (HA-PADM) and Gelfoam at day 1, day 3, and day 6. The low magnification images (200×) showed HA-PADM has a tightened and porous structure, while Gelfoam has a loose and porous structure. The high magnification images (1000×) were enlarged from the rectangle area in the low magnification images. Cells (showed with arrows) on HA-PADM have multiple processes (showed with arrowheads) and connected to each other. Multiple layers of cells were formed on day 3 and day 6 on the surface of HA-PADM scaffolds. Gelfoam presents a smooth surface and a single layer of cells (showed with arrows) firmly attached to the Gelfoam surface. The shapes of cells were flat with microfiber-like process.

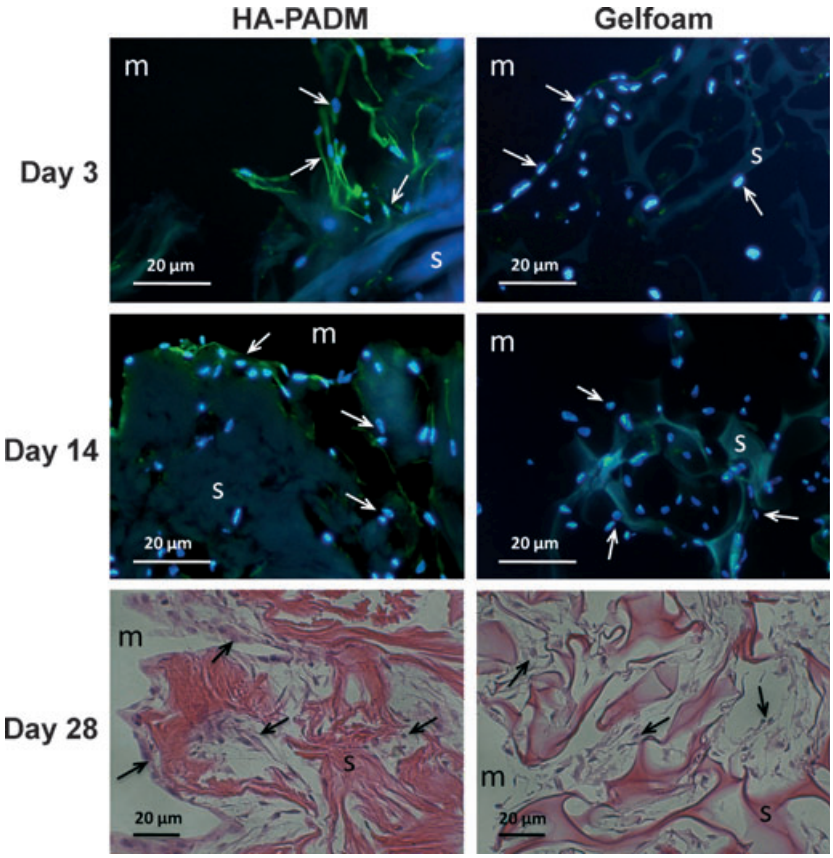

Fig. 4. Human periodontal ligament (hPDL) cell migration in hydroxyapatite–porcine acellular dermal matrix (HA-PADM) and Gelfoam. The images of cell migration were shown in the frozen cross-sections of the samples at day 3 and day 14 with immunohistochemical staining and at day 28 with hematoxylin and eosin (H&E) staining. At day 3, hPDL cells (showed with arrows) form multiple layers on the surface of HA-PADM with fewer cells grow into the scaffold; but a layer of the cells attached on and fewer cells stay inside of the Gelfoam. The F-actin fibers were stained in green and nuclei in light blue. At day 14, more cells were presented inside of the scaffold suggesting hPDL cells could migrate into the scaffolds. Strong F-actin expression detected for the cells cultured with HA-PADM, while it is hardly present for the cells cultured with Gelfoam. The F-actin fibers were stained in green and nuclei in light blue. At day 28, H&E staining showed hPDL cells (showed with arrows) had grown inside both the Gelfoam and the HA-PADM scaffold. M represents the medium side of the scaffold; S represents the scaffold part of the cross-section.

high mechanical strength is important for biodegradable scaffolds (13–15) or allogenic acellular matrix scaffolds (16). Major disadvantages attributable to biodegradable polymer scaffolds include the need to reconstruct the natural three-dimensional structures, and the absence of important extracellular matrix (ECM) proteins in the synthetic polymers (17). Thus, acellularized xenogenic tissues that maintain natural 3-dimensional structures are a promising alternative to biodegradable polymer scaffolds (18–20). PADM, which is mainly composed of type I and II collagen, has been used successfully in experimental, and clinical soft tissue reconstruction (21–24). Comparison study showed that human and PADM were similar in terms of histological structure, ultrastructure of collagen fiber, variety and disposition of main protein and biocompatibility (8). Although animal studies found immune rejection responses associated with porcine-derived organ transplantation (25), long-term clinical follow-up studies showed successful tissue reconstruction with the use of PADM in skin grafting (7, 26). The modifications in PADM preparation could also greatly reduce xenogeneic antigens to minimize immune response (21).

In periodontal therapy, a biodegradable scaffold is required to deliver cells to the wound site, as well as for preserving the space of the defect during the new periodontal tissue formation. The periodontium consists of both soft and hard tissues while a pure collagen framework lacks calcium and has a weak initial com-

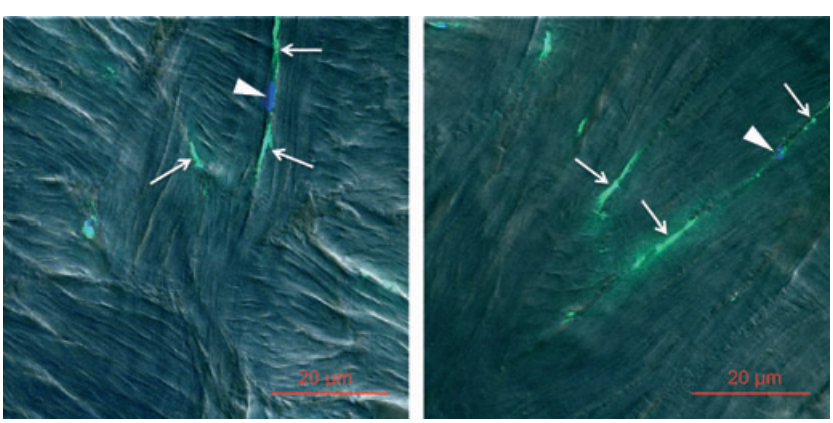

Fig. 5. Reconfirmation of human periodontal ligament (hPDL) cell migration in hydroxyapatite–porcine acellular dermal matrix (HA-PADM). Confocal microscopy imaging at day 6 reviewed that the hPDL cells had grown inside the scaffold and spread along the direction of the collagen fibers. Arrows pointed the F-actin stained in green, showing the cell bodies stretched along the collagen fibers. Arrowheads pointed the nuclei stained in blue.

pressive strength and thus cannot be used for tissue regeneration. HA the main inorganic component of bone and teeth, has been widely used in dental implant materials because of its biocompatibility and osteogenic potential (27). In this study, we evaluated the biocompatibility of a novel HA-modified porcine acellular dermal scaffold (HA-PADM) for periodontal tissue regeneration. Using hPDL cells, we tested the suitability of the scaffolds in periodontal tissue regeneration. The HA-PADM scaffold prepared by a two-step biomimetic mineralization method maintained the natural 3D microstructure of the tissue while the HA coating reduced the degradation rate and improved the mechanical property of the collagen scaffold (5). Moreover, the HA-PADM proved to be hydrophilic when we drop the cell suspension on the dried materials. All these properties make HA-PADM highly promising as a material for periodontal tissue regeneration.

From the cell proliferation assay, we found that cells grow relatively well with Gelfoam, a commercially available gelatin sponge. SEM imaging showed Gelfoam has a loose porous structure compare to a HA-PADM scaffold, so that it provides more space for cellular migration and proliferation. However, on the smooth microsheet structures of Gelfoam, hPDL cells were flat and firmly attached to the surface of the material in comparison with the cells seen on HA-PADM that had more processes and connected with each other in three-dimensions. Importantly, Gelfoam does not have the required biomechanical stiffness that HA-PADM has, to maintain a proper shape for periodontal regeneration. Because we also observed higher polymerized filamentous actin (F-actin) expression levels in cells on HA-PADM, we believe that the cell behavior with HA-PADM scaffold more closely approximates its natural state. Furthermore, the biodegradation rate of Gelfoam is not optimized for periodontal regeneration. With prolonged culture for 4 weeks, it was clearly observed that the PDL cells were able to migrate into the center of the HA-PADM scaffold, and in some places, fill the channels in scaffolds.

In this *in vitro* culture system, we could not include the factor of biodegradation, which is very important for the process of tissue regeneration. With the presence of collagenase and other enzymes in the microenvironment of the peridontium, the condensed structure of HA-PADM could be at an advantage in terms of tissue regeneration. With further modifications of the HA-PADM scaffold to provide more openings for cells to grow into, it is possible that HA-PADM could be used as a cell carrier for periodontal tissue regeneration. The next phase of the investigation will involve *in vivo* evaluation of the HA-PADM scaffold in terms of its ability to deliver cells, maintain space, and to offer timely degradation in periodontal defects.

In conclusion, the concepts of periodontal regeneration research demand tissue engineering with high cytocompatibility, guiding channel-like structures and biodegradable scaffolds. Our study shows that hPDL cells proliferate and migrate into the novel HA-PADM scaffold. The cell behavior was positively influenced by the scaffold. The properties of this acellularized, HA-treated porcine collagen scaffold (HA-PADM) makes it a promising biomaterial for use as a cell carrier in periodontal regeneration.

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Conflict of interest

The authors clarify that there is no conflict of interest for this study and publication.

References

1. Brown LJ, Loe H. Prevalence, extent, severity and progression of periodontal disease. Periodontol 2000 1993;2:57–71.
2. Papapanou PN. Epidemiology of periodontal diseases: an update. J Int Acad Periodontol 1999;1:110–6.
3. Chen FM, Jin Y. Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. Tissue Eng Part B Rev 2010;16:219–55.
4. Hughes FJ, Ghuman M, Talal A. Periodontal regeneration: a challenge for the tissue engineer? Proc Inst Mech Eng H 2010;224:1345–58.
5. Giannopoulou C, Cimasoni G. Functional characteristics of gingival and periodontal ligament fibroblasts. J Dent Res 1996;75:895–902.
6. Wang HL, Greenwell H, Fiorellini J, Giannobile W, Offenbacher S, Salkin L, et al. Periodontal regeneration. J Periodontol 2005;76:1601–22.
7. Jiong C, Jiake C, Chunmao H, Yingen P, Qiuhe W, Zhouxi F et al.Clinical application and long-term follow-up study of porcine acellular dermal matrix combined with autoskin grafting. J Burn Care Res 2010;31:280–5.
8. Ge L, Zheng S, Wei H. Comparison of histological structure and biocompatibility between human acellular dermal matrix (ADM) and porcine ADM. Burns 2009;35:46–50.
9. Zhao H, Wang G, Hu S, Cui J, Ren N, Liu D, et al. In vitro biomimetic construction of hydroxyapatite-porcine acellular dermal matrix composite scaffold for MC3T3-E1 preosteoblast culture. Tissue Eng Part A 2011;17:765–76.
10. Kramer PR, Nares S, Kramer SF, Grogan D, Kaiser M. Mesenchymal stem cells acquire characteristics of cells in the periodontal ligament in vitro. J Dent Res 2004;83:27–34.
11. Lee CH, Singla A, Lee Y. Biomedical applications of collagen. Int J Pharm 2001;221:1–22.
12. Ma L, Gao C, Mao Z, Zhou J, Shen J. Enhanced biological stability of collagen porous scaffolds by using amino acids as novel cross-linking bridges. Biomaterials 2004;25:2997–3004.
13. Hoerstrup SP, Sodian R, Daebritz S, Wang J, Bacha EA, Martin DP, et al. Functional living trileaflet heart valves grown in vitro. Circulation 2000;102:III44–9.
14. Shinoka T, Shum-Tim D, Ma PX, Tanel RE, Isogai N, Langer R, et al. Creation of viable pulmonary artery autografts through tissue engineering. J Thorac Cardiovasc Surg 1998;115:536–45; discussion 45-6.

15. Stock UA, Nagashima M, Khalil PN, Nollert GD, Herden T, Sperling JS, et al. Tissue-engineered valved conduits in the pulmonary circulation. J Thorac Cardiovasc Surg 2000;119: 732–40.
16. Steinhoff G, Stock U, Karim N, Mertsching H, Timke A, Meliss RR, et al. Tissue engineering of pulmonary heart valves on allogenic acellular matrix conduits: in vivo restoration of valve tissue. Circulation 2000;102:III50–5.
17. Joshi P, Chung CY, Aukhil I, Erickson HP. Endothelial cells adhere to the RGD domain and the fibrinogen-like terminal knob of tenascin. J Cell Sci 1993;106:389–400.
18. Tamura N, Nakamura T, Terai H, Iwakura A, Nomura S, Shimizu Y, et al. A new acellular vascular prosthesis as a scaffold for host tissue regeneration. Int J Artif Organs 2003;26:783–92.
19. Lin CC, Ritch R, Lin SM, Ni MH, Chang YC, Lu YL, et al. A new fish scale-derived scaffold for corneal regeneration. Eur Cell Mater 2010;19:50–7.
20. Yang Z, Shi Y, Wei X, He J, Yang S, Dickson G, et al. Fabrication and repair of cartilage defects with a novel acellular cartilage matrix scaffold. Tissue Eng Part C Methods 2010;16:865–76.
21. Xu H, Wan H, Zuo W, Sun W, Owens RT, Harper JR, et al. A porcine-derived acellular dermal scaffold that supports soft tissue regeneration: removal of terminal galactose-alpha-(1,3)-galactose and retention of matrix structure. Tissue Eng Part A 2009;15:1807–19.
22. Herford AS, Akin L, Cicciu M, Maiorana C, Boyne PJ. Use of a porcine collagen matrix as an alternative to autogenous tissue for grafting oral soft tissue defects. J Oral Maxillofac Surg 2010;68:1463–70.
23. Badhe SP, Lawrence TM, Smith FD, Lunn PG. An assessment of porcine dermal xenograft as an augmentation graft in the treatment of extensive rotator cuff tears. J Shoulder Elbow Surg 2008;17:35S–9S.
24. Hsu PW, Salgado CJ, Kent K, Finnegan M, Pello M, Simons R, et al. Evaluation of porcine dermal collagen (Permacol) used in abdominal wall reconstruction. J Plast Reconstr Aesthet Surg 2009;62:1484–9.
25. DeSagun EZ, Botts JL, Srivastava A, Hanumadass M, Walter RJ. Long-term outcome of xenogenic dermal matrix implantation in immunocompetent rats. J Surg Res 2001;96: 96–106.
26. Pan YC, Xu JQ, Chen YK, Li SL, Chen SH, Wu DN, et al. The clinical application on composite graft of acellular allodermal matrix and auto-microskin on escharectomy after deep burns. Zhonghua Wai Ke Za Zhi 2004;42:410–2.
27. Ong JL, Chan DC. Hydroxyapatite and their use as coatings in dental implants: a review. Crit Rev Biomed Eng 2000;28:667–707.