Periodontal healing using a collagen matrix with periodontal ligament progenitor cells in a dehiscence defect model in beagle dogs

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Periodontal healing using a collagen matrix with periodontal ligament progenitor cells in a dehiscence defect model in beagle dogs

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ABSTRACT

Periodontal healing using a collagen matrix with periodontal ligament progenitor cells in a dehiscence defect model in beagle dogs

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Objectives: To histologically characterize periodontal healing at 8 weeks in surgically created dehiscence defects in beagle dogs that received a collagen matrix with periodontal ligament (PDL) progenitor cells.

Materials and methods: The bilateral maxillary premolars and first molars in 6 animals were used. Standardized experimental dehiscence defects were made on the buccal side of 3 premolars, and primary culturing of PDL progenitor cells was performed on the molars. Collagen matrix was used as a scaffold and a delivery system for PDL progenitor cells. The experimental sites were grafted with collagen matrix (COL), PDL progenitor cells with collagen matrix (COL/CELL), or

left without any material (CTL). Histologic and histomorphometric analyses were performed after 8 weeks.

Results: The defect height from the cementoenamel junction to the most apical point of cementum removal did not significantly different across the CTL, COL, and COL/CELL groups, at 4.57±0.28, 4.56±0.41, and 4.64±0.27 mm (mean ± standard deviation), respectively; the corresponding values for epithelial adhesion were 1.41±0.51, 0.85±0.29, and 0.30±0.41 mm (P<0.05), the heights of new bone regeneration were 1.32±0.44, 1.65±0.52, and 1.93±0.61 mm (P<0.05), and the cementum regeneration values were 1.15±0.42, 1.81±0.46, and 2.57±0.56 mm (P<0.05). There was significantly more new bone formation in the COL/CELL group than in the CTL group, and new cementum length was also significantly higher in the COL/CELL group. However, there were not significantly different in the width of new cementum among the groups.

Conclusion: PDL progenitor cells carried by a synthetic collagen matrix may enhance periodontal regeneration, including cementum and new bone formation.

Keywords: Collagen matrix; Dehiscence defect; Periodontal ligament progenitor cells; Periodontal regeneration

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I. Introduction

The goal of root coverage procedures in periodontal surgery is to regenerate the periodontal supporting tissues, such as connective-tissue attachments, cementum, and the alveolar bone. However, conventional treatments yield a wide spectrum of outcomes, ranging from the formation of new attachments to the formation of long junctional epithelia (Melcher 1976). Guided tissue regeneration has been considered the most successful of these conventional periodontal treatment modalities, since it provides space for the ingrowth of progenitor cells with the use of a barrier membrane. Even though excellent preclinical and clinical outcomes in resolving intrabony and furcation defects have demonstrated the successful results that can be obtained by using guided tissue regeneration, the sensitivity of this technique has also produced a wide range of clinical

outcomes, including membrane exposure and postoperative infections resulting in the severe loss of periodontal structures (Wikesjo, Kean et al. 1994, Han, Menicanin et al. 2014).

Novel research methods using progenitor cells have also produced histologic evidence of the formation of new periodontal attachments, albeit with some limitations (Liu, Zheng et al. 2008, Anitua, Orive et al. 2009, Kunze, Huber et al. 2009, Lin, Gronthos et al. 2009, Thoma, Hammerle et al. 2011, Tsumanuma, Iwata et al. 2011, Thoma, Villar et al. 2012, Iwasaki, Komaki et al. 2013). In our previous *in vivo* experiment, periodontal attachments between bone and dental root surfaces were formed *de novo* in a replanted tooth model by directly adhering cultured periodontal ligament (PDL) progenitor cells onto the root surface (Lee, Kim et al. 2015). However, this regenerated tissue clearly differed from the pristine PDL in terms of both its thickness and density, and in the orientation and insertion into both sides of the bone and root cementum. No previous study has utilized an appropriate methodology or scaffolding biomaterial for carrying cells, which might explain the aforementioned limitations.

Collagen matrix has recently been introduced in clinical applications for focused indications of root coverage and gingival augmentation based on its ability to provide space for soft-tissue augmentation, as demonstrated in several preclinical studies (Bartold, Xiao et al. 2006, Ding, Liu et al. 2010, Seo, Thoma et al. 2018). Collagen matrix is a candidate carrier of progenitor cells in dehiscence defects, such as in root coverage techniques with the goal of achieving genuine periodontal regeneration, rather than simply periodontal repair.

With this theoretical background, it was hypothesized that PDL progenitor cells carried by a collagen substitute can promote the formation of periodontal attachments at periodontally impaired root surfaces. Therefore, the purpose of this study was to obtain histologic measurements of periodontal healing after applying a collagen matrix with or without PDL progenitor cells to a dehiscence defect model in beagle dogs.

II. Materials and Methods

1. Materials

1.1 Animals

Six male beagle dogs (15–20 months old, weighing 10 kg) with complete dentition and a sound periodontium were used in this study. The protocols for animal selection, supervision, preparation and the surgical protocols were approved by the Animal Care and Use Committee of Yonsei Medical Center, Seoul, Korea (Approval No. 2015-0233).

1.2 PDL progenitor cell culture

We used the bilateral maxillary first molars for the primary culture of PDL progenitor cells. After meticulously removing dental plaque and calculus and applying iodopovidone, the teeth were sectioned and then gently extracted under combined general and local anesthesia. The isolation and culturing of PDL progenitor cells were performed using protocols that we have described previously (Lee, Kim et al. 2015). In summary, the PDL was carefully isolated from the root surface at the apical and middle regions of the dental roots using a scalpel, and ground the tissue into pieces that were as small as we could. The ground PDL was dissolved five times with 30 minutes period in α-Minimum Essential Medium (α-MEM; Gibco, NY, USA) including 100 U/mL type I collagenase (Wako, Tokyo, Japan) and 2.5 U/mL dispase (Gibco) at 37°C. Single cell suspensions were acquired carrying by a strainer with a 70 μm pore size (Falcon, BD Labware, NJ, USA). 5×10^5 cells were scattered on cell-culture flasks (T75, BD Biosciences, NJ, USA) including α-MEM with the addition of 100 μM L-ascorbic acid 2-phosphate (Sigma-Aldrich, MO, USA), 2

μM L-glutamine (Gibco), 100 μg/mL streptomycin (Gibco), 100 U/mL penicillin and 15% fetal bovine serum (FBS; Gibco). After that it was cultivated at 37°C with 5% CO_2 atmosphere. After three to seven days, single-cell colonies were discovered and we cultivated PDL progenitor cells at passage P0. We used P3 and P4 cells for experiment.

1.3 Characteristics of isolated PDL progenitor cells

Colony-forming-units assay

We plated the cells at 1×10^3 cells/10 mL on the 100-mm culture dishes (Nunc; Thermo Scientific, Waltham, MA, USA) with growth medium. The fixation was conducted with 4% formaldehyde. The staining was performed with crystal violet (Sigma-Aldrich). At 14 days after seeding, we observed with light microscopy (CK40; Olympus Optical, Tokyo, Japan).

Osteogenic and adipogenic differentiation

We scattered the cells in 6-well plates (Nunc; Thermo Scientific) with the density of 1×10^5 cells/well and we cultivated since they became subconfluent condition. Osteogenic differentiation culture medium is composed of α-MEM including 2 mM β-glycerophosphate (Sigma-Aldrich), 10^{-8} M dexamethasone (Sigma-Aldrich), 1.8 mM KH_2PO_4 (Sigma-Aldrich), 2 mM L-glutamine (Gibco), 15% FBS (Gibco), 100 U/mL penicillin (Gibco), 100 mg/ml streptomycin (Gibco), 100 mM L-ascorbic acid 2-phosphate and 55 mM 2-mercaptoethanol (AMRESCO, Solon, OH, USA). Those were freshened with 3 days periods. Adipogenic differentiation culture medium is composed of α-MEM 5 mM hydrocortisone (Sigma-Aldrich), 60 mM indomethacin (Sigma-Aldrich), 500 mM isobutyl-methylxanthine (Sigma-Aldrich), 10 mM insulin (Sigma-Aldrich), 100 mM L-ascorbic acid 2-phosphate, 15% FBS, 2 mM L-glutamine, 100 mg/mL streptomycin and

100 U/mL penicillin. Osteogenic differentiation was obtained after 2 weeks and adipogenic differentiation was obtained after 4weeks. The osteogenic differentiation staining of the cells were performed with alizarin red (Sigma-Aldrich) and adipogenic differentiation of the cells were with Oil Red O (Sigma-Aldrich).

1.4 Collagen matrix as a carrier of PDL progenitor cells

The collagen matrix (20 mm × 40 mm × 2 mm; Collagen Graft, Genoss, Suwon, Korea) consisted of 2 pure bovine collagen layers differing in density: a thick spongious layer and a thin compact layer (Seo, Thoma et al. 2018). For evaluating progenitor cell adhesion, scanning electron microscopy (SEM; S-300N, Hitachi, Tokyo, Japan) was applied after a short period of incubation (0, 10, or 30 minutes) following cell seeding (1×10^6 cells) to a collagen matrix of standardized size (10 mm × 10 mm).

2. Study design and protocols

A surgically created dehiscence model was used, and the following 3 experimental groups were constructed:

- Sham control (CTL) group, in which a dehiscence defect was induced but had no biomaterial grafted into it.
- Collagen matrix (COL) group, in which a dehiscence defect was induced and grafted with collagen matrix.
- PDL progenitor cells with collagen matrix (COL/CELL) group, in which a dehiscence defect was induced and grafted with PDL progenitor cells carried by a collagen matrix.

The bilateral first, second, and third maxillary premolars of the 6 beagle dogs were involved in this experiment, and the 3 groups were allocated rotationally to ensure an even distribution of experimental sites for each group. Single roots of the first premolar and both mesial and distal roots of the second/third premolars were included, but the individual teeth were involved as the experimental unit for each group. The numerical distribution of the involved teeth was even for each group, and 12 teeth (20 dental roots) were included in 6 animals.

After elevating a full-thickness mucoperiosteal flap in the experimental region, dehiscence defects with a standardized size (5 mm long) were surgically created in all dental roots of the included premolars. Alveolar bone was carefully removed using a diamond bur attached to a high-speed rotary tool to expose the dental root surface over a length of 5 mm from the cementoenamel junction (CEJ), and the surface was finally planed using hand-operated instruments to completely remove the pre-existing cementum. The experimental biomaterial was applied onto the dehiscence defect area as appropriate for the allocated group for each site, with collagen matrix trimmed to the standardized size (10 mm × 10 mm) in the COL and COL/CELL groups, and incubated for 30 minutes after seeding 1×10^6 PDL progenitor cells on the collagen matrix in the COL/CELL group. The mucoperiosteal flap was relocationed and sutured closely at the CEJ, enveloping the grafted materials applying a monofilament nylon suture (Monosyn, B. Braun, Melsungen, Germany) (Figure 1). A widespread intramuscularly antibiotic (cefazoline sodium [20 mg/kg]; Yuhan, Seoul, Korea) were given to the dogs. And for fourteen days 0.2% chlorhexidine solution (hexamidine; Bukwang Pharmaceutical, Seoul, Korea) was received. We removed the monofilament nylon suture material after 7 days of surgery.

3. Histologic processing

After 8 weeks of surgery the dogs were sacrificed applying an overdose of pentobarbital sodium (90–120 mg/kg intravenously; Entobar; Hanlim Pharmaceutical, Seoul, Korea). The fixation of the study sites block sections were performed for ten days with 10% neutral buffered formalin. All the sites of experimental and control groups were dissected along with the surrounding soft and hard tissues. The specimens were demineralized with a specific solution containing formic acid (Rapid-Cal; BBC Biochemical, Mount Vernon, WA, USA), trimmed, dehydrated, and embedded in paraffin. Serial sections with a thickness of 6 μm were prepared in the buccolingual plane. Staining was performed with hematoxylin-eosin and Masson trichrome.

4. Histologic and histomorphometric analysis

Histologic and histometric analyses were carried out applying both incandescent- and polarized-light microscopy (BX41, Olympus, Tokyo, Japan) and a PC-based image analysis system (Adobe Photoshop CS3, Adobe Systems, CA, USA). The bottom of the defect was set at the most apical point of the planed root surface where the preexisting cementum had been removed, and the following measurements were made (Figure 2):

- Defect height, corresponding to the distance between the bottom of the defect and CEJ.
- New cementum length, corresponding to the distance from the bottom of the defect to the most coronal point of newly formed cementum on the root surface.
- New cementum width, corresponding to the average width of newly formed cementum measured at 3 different levels: the most coronal, most apical, and middle levels of the

whole length of newly formed cementum.

- New bone, corresponding to the distance from the bottom of the defect to the most coronal point of newly formed bone alongside the root surface.
- Connective-tissue adhesion, corresponding to the distance from the most apical point of the long junctional epithelium to the most coronal point of the new cementum.
- Epithelial adhesion, corresponding to the distance from the most apical point of the long junctional epithelium to the CEJ.

5. Statistical analysis

The statistical analysis was accomplished with a standard software program (SPSS version 25.0; IBM Corp., Armonk, NY, USA). We applied One-way analysis of variance and the Tukey *post hoc* multiple-comparisons test to detect significant differences between the groups. The Kolmogorov-Smirnov test confirmed the normality of the distribution of the data ($P>0.05$). Statistical tests were performed with an alpha level of 0.05. Measured data are presented as mean ± standard deviation.

III. Results

1. Characterization of PDL progenitor cells

After seven to fourteen days we observed the adherent clonogenic clusters of fibroblast-like cells at single-cell suspension that was obtained by PDL of animals. This certifies the multipotent capacity of the PDL progenitor cells. We observed long spindle-like fibroblastic cells after seven to fourteen days of cell plating and 60–100 single colonies were obtained from 1×10^3 single cells. P4 cells also produced high number of single colonies on culture plates despite the fact that the high passage number produced the result of decreased single colony number. Osteogenic differentiation was observed with alizarin-red–positive cells and adipogenic differentiation of the cells was observed with Oil-Red-O–positive cells. This demonstrates the proof of multipotency of PDL progenitor cells (Figure 3).

2. SEM analysis of collagen matrix seeded with PDL progenitor cells

Cell adhesion to the collagen matrix was observed by SEM at 0, 10, and 30 minutes after seeding PDL progenitor cells. Cuboidal cells were dispersed among a network of collagen fibrils, and a fine microfibrous network in which the cells were caught and maintained could be observed on samples incubated for 10 minutes. This network was increasingly observed on the samples incubated for 30 minutes, and cellular surface irregularities indicating initial cellular processes were evident. Although no cellular processes clearly attached onto the surfaces of the collagen

matrix, most of the cells were stably maintained with the microfibrous network (Figure 4).

3. Clinical observations

All study sites were healed without any event. There were no adverse effects such as infection, root resorption. After surgical experiment the healing was equivalent in the control and experimental groups.

4. Histologic and histomorphometric results

All sites in the 6 animals showed the newly formed bone and cementum alongside the surgically created tooth dehiscence defects (Figure 5 and 6). No inflammatory cell infiltration was observed at the sites of all animals, and there was limited evidence of a collagen matrix at all test sites. There was no significant intergroup difference in the defect height from the CEJ to the most apical point of cementum removal (4.57±0.28, 4.56±0.41, and 4.64±0.27 mm in the CTL, COL, and COL/CELL groups, respectively). The sulcular depth with epithelial adhesion from the CEJ was smaller in the test sites that received collagen and PDL progenitor cells, and the corresponding epithelial adhesion values differed significantly across the CTL, COL, and COL/CELL groups (1.41±0.51, 0.85±0.29, and 0.30±0.41 mm, respectively; $P<0.05$); however, there were no significant differences in connective-tissue adhesion, with values of 2.01±0.11, 1.90±0.14, and 1.77±0.40 mm, respectively.

Newly formed bone appeared to have grown from the base of the defect, and also had the appearance of immature woven bone with limited lamellation and no osteon formation. These

patterns were shown in all experimental groups without significant differences. The heights of new bone regeneration were 1.32±0.44, 1.65±0.52, and 1.93±0.61 mm in the CTL, COL, and COL/CELL groups, respectively. The COL/CELL group showed significantly greater new bone formation than the CTL group (P<0.05), but there were no qualitative differences in newly formed bone between the groups. The PDL was shaped across the newly formed bone and the surface of the dental root. And it is shown that collagen fibers are inserted perpendicularly into the newly formed bone and cementum(Figure 6).

New cementum was formed on the exposed dental root, dentin surfaces, and appeared as a layer of mineralized tissue with or without cellular impaction within the layer. The length of new cementum regeneration varied across the CTL, COL, and COL/CELL groups (at 1.15±0.42, 1.81±0.46, and 2.57±0.56 mm, respectively; P<0.05). However, the intergroup differences in the width of new cementum were not statistically significant, with values of 11.66±3.23, 12.80±3.43, and 14.44±2.98 μm, respectively. Table 1 and Figure 7 summarize the histometric results.

IV. Discussion

The purpose of this study was to conduct a histologic assessment of the recovery of periodontal tissue healing after grafting a collagen matrix with PDL progenitor cells on surgically created dehiscence bone defects in beagle dogs. The PDL progenitor cells carried by the collagen matrix were found to significantly enhance periodontal regeneration, including the growth of cementum and alveolar bone. This result can be interpreted in light of recent findings that the multipotential capacity of PDL progenitor cells enhanced periodontal regeneration, including cementum formation (Akizuki, Oda et al. 2005, Hasegawa, Yamato et al. 2005, Gronthos, Mrozik et al. 2006, Flores, Yashiro et al. 2008, Iwata, Yamato et al. 2009, Kim, Kim et al. 2009, Feng, Akiyama et al. 2010). Seo et al. (Seo, Miura et al. 2004) reported that the multipotential capacity of PDL progenitor cells from human PDL could enhance periodontal tissue regeneration. Those authors found that PDL progenitor cells could differentiate into cementoblast-like cells, adipocytes, osteocytes, and collagen-forming cells. When transplanted into immunocompromised rodents, PDL progenitor cells showed the ability to formulate a cementum/PDL-like tissue and contributed to periodontal tissue regeneration.

Several animal studies also have provided evidence that PDL progenitor cells can safely and effectively enhance periodontal regeneration (Volponi, Pang et al. 2010, Suaid, Ribeiro et al. 2012, Mrozik, Wada et al. 2013, Yu, Oortgiesen et al. 2013). Akizuki et al. (Akizuki, Oda et al. 2005) applied PDL cell sheets with a reinforced hyaluronic acid carrier to surgically created dehiscence defects in beagle dogs. That study observed periodontal tissue regeneration containing bone, PDL, and cementum in the experimental group, and found that there was significantly more newly formed cementum in the experimental group than in the group with only the hyaluronic acid

carrier. Murano et al. (Murano, Ota et al. 2006) transplanted PDL progenitor cells into class III furcation defects in dogs, and demonstrated that new cementum regenerated around the entire root surfaces of the class III furcation, with new bone filling , whereas the no-treatment group showed reduced epithelial growth and furcation defects filled with connective tissue.

Successful periodontal regeneration requires the utilization and recruitment of progenitor cells that can differentiate into specialized cells with a regenerative capacity, followed by the proliferation of these cells and the synthesis of specialized materials that can maintain the required space (Bartold, Xiao et al. 2006). Collagen matrix is a scaffolding material for soft-tissue augmentation that was originally developed as a replacement of an autogenous connective-tissue graft, and it can provide space to stimulate the growth of fibroblasts and blood vessels. It was reported by Mathes et al. (Mathes, Wohlwend et al. 2010) that prototype collagen matrices *in vitro* showed primary human fibroblast growth and the expression of extracellular matrix proteins such as collagen type I, as well as an increase in fibronectin. The application of collagen matrix allows the regenerative ability of cells existing within the periodontium to be utilized.

In the present study, we applied collagen matrix with PDL progenitor cells to surgically created dehiscence defects in beagle dogs. The histometric analyses showed that the formation of cementum in the group that received PDL progenitor cells with collagen matrix was significantly greater than that in the sham control and collagen matrix groups. Functional insertion of collagen fibers into the newly formed cementum was found evenly in all samples from all 3 experimental groups. The histometric analyses also suggested that newly formed bone was significantly greater in the group with PDL progenitor cells and collagen matrix than in the sham control group. The result suggested that PDL progenitor cells have a multipotential ability that contributes to the regeneration of periodontal tissue and implies that a collagen matrix can facilitate cell adhesion and proliferation and provide room for space management.

An important issue in this study was the outcome of the transplanted cells and the effectualness of the cell delivery system. SEM revealed stably clustered PDL progenitor cells that were enmeshed in the web of the collagen fiber network, with few cellular tentacles. Even though more time for cellular attachment and other metabolic cycles could clearly enhance the delivery of PDL progenitor cells, only a short period of cellular application was used due to the vulnerability of the collagen matrix to resorption within the cellular medium (Seo, Thoma et al. 2018). Despite the short time of cellular application, periodontal regeneration was clearly enhanced in the *in vivo* experiment, and we also obtained evidence of its advantages as an optimal cell delivery system for periodontal healing.

Within the limitations of this study, the present results suggest that PDL progenitor cells carried on a synthetic collagen matrix can enhance periodontal regeneration, including cementum formation and new bone formation, even with a protocol involving a short period of cell application (30 minutes). Collagen matrices should therefore be considered as a promising candidate carrier for PDL progenitor cells in periodontal tissue engineering.

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Table

Table 1. Results of histometric analyses of periodontal tissue formation after surgery (one-way analysis of variance).

	Sham control	Collagen matrix	PDL progenitor cells with collagen matrix
Defect height	4.57±0.28	4.56±0.41	4.64±0.27
New bone	1.32±0.44	1.65±0.52	1.93±0.61*
New cementum length	1.15±0.42†	1.81±0.46	2.57±0.56*,†
New cementum width¶	11.66±3.23	12.80±3.43	14.44±2.98
Connective-tissue attachment	2.01±0.11	1.90±0.14	1.77±0.40
Epithelial adhesion	1.41±0.51†	0.85±0.29	0.30±0.41*,†

PDL, periodontal ligament
Data are mean±SD values, in millimeters
¶Data are mean±SD values, in micrometers
*$p<0.05$ compared with sham control
†$p<0.05$ compared with collagen matrix

Figure Legends

Figure 1. Clinical photographs of the surgical procedure. (A) Preoperation view of first, second, and third premolars. (B) The dehiscence defect with a size of 5 mm × 5 mm (width × length) was surgically created. (C) Each site of a dehiscence defect was grafted with collagen matrix with periodontal ligament (PDL) progenitor cells (COL/CELL group), grafted with collagen matrix alone (COL group), or left without any material (CTL group). (D) Primary closure was then performed.

Figure 2. Schematic drawing of measurements. The bottom of the defect was set at the most-apical point of the planed root surface, the most-coronal point of newly formed cementum on the root surface was marked by a straight line and the most-apical point of the long junctional epithelium was also marked by a straight line. The newly formed bone was distinguished from the preexisting alveolar bone by their colors. B, bone; PDL, periodontal ligament; C, cementum; JE, junctional epithelium; DH, defect height; NC, new cementum; CTA, connective-tissue adhesion; EA, epithelial adhesion; NB, new bone

Figure 3. Characterization of PDL progenitor cells as MSCs. (A, B) Colony-forming-units assay revealed colony formation at 14 days after cell plating (stained with crystal violet). Although the colony formation decreased from passage (P)3 (A) to P4 (B), the size of the colonies was similar at P3 and P4 (C, D). Adipogenic and osteogenic differentiation were induced after 4 and 2 weeks, respectively. Scale bar = 100 μm in (C), (D).

Figure 4. Scanning electron microscopy photographs showing collagen matrix with PDL progenitor cells in the experimental group at observation periods of 0 minutes (A), 10 minutes (B), and 30 minutes (C). The number of cells tended to decrease from 0 to 30 minutes, with some cells showing a spindle-like appearance.

Figure 5. Photomicrographs of the buccolingual section in the dehiscence defects of periodontal tissue in the CTL (A), COL (B), and COL/CELL (C) groups (scale bar=400 μm), displaying the sites from the apical extension of the surgically created defect (apical insert; a, d, and g) along the root surface to the coronal extension of the newly formed alveolar bone and cementum (midroot insert; b, e, and h), and the apical extension of epithelial adhesion (c, f, and i). JE, junctional epithelium; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum (hematoxylin-eosin stain, scale bar=200 μm).

Figure 6. Low magnification photograph of the buccolingual section in the dehiscence defects of periodontal tissue in the CTL (a), COL (b), and COL/CELL (c) groups (scale bar=300 μm). And high magnification photograph from sites of the dehiscence defects of periodontal tissue in the CTL (A), COL (B), and COL/CELL (C) groups. The newly formed PDL did not differ between the three groups. AB, alveolar bone; PDL, periodontal ligament; Ce, cementum (hematoxylin-eosin stain, scale bar = 100 μm).

Figure 7. Comparison of periodontal tissue formation. (A) The defect height did not differ significantly between the three groups ($p=0.84$). (B) New bone formation did not differ significantly between the three groups ($p=0.058$). However, Tukey's post-hoc test revealed a significant difference between the CTL and COL/CELL groups ($p=0.046$). (C) New cementum formation differed significantly between the three groups ($p=0.001$). *$p < 0.05$.

Figures

Figure 1

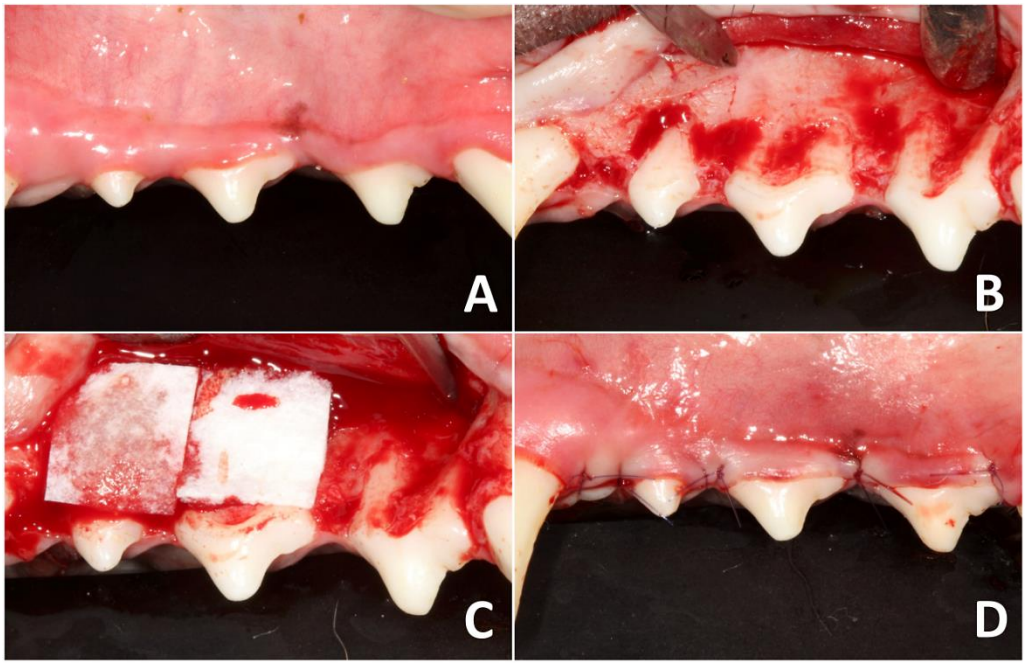


Figure 2

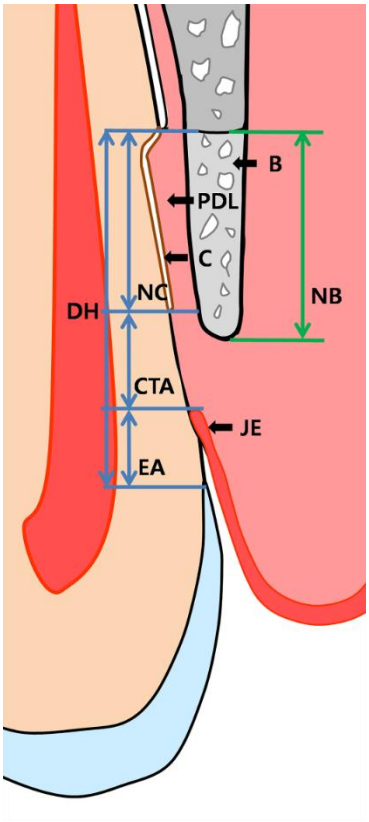


Figure 3

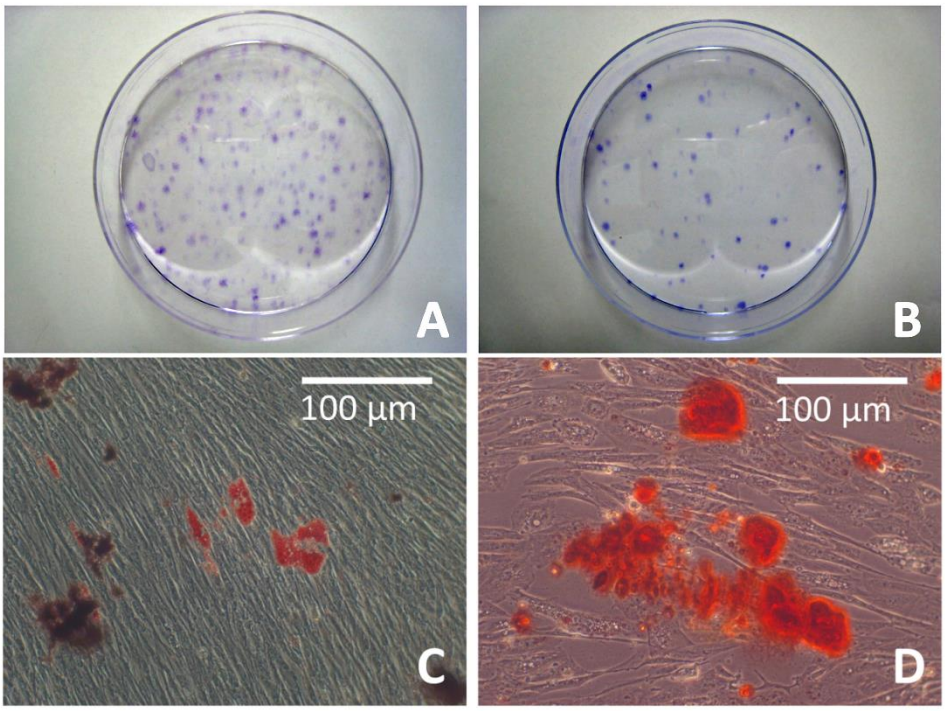


Figure 4

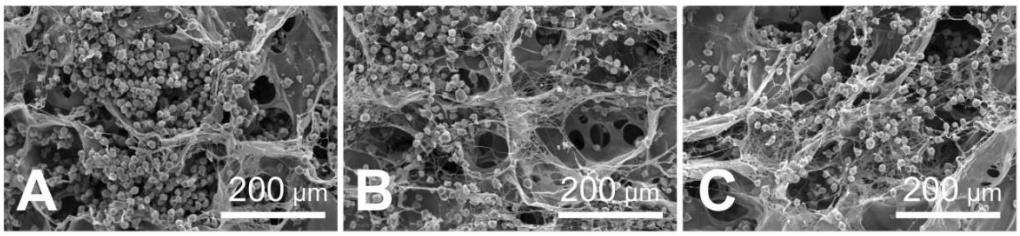


Figure 5

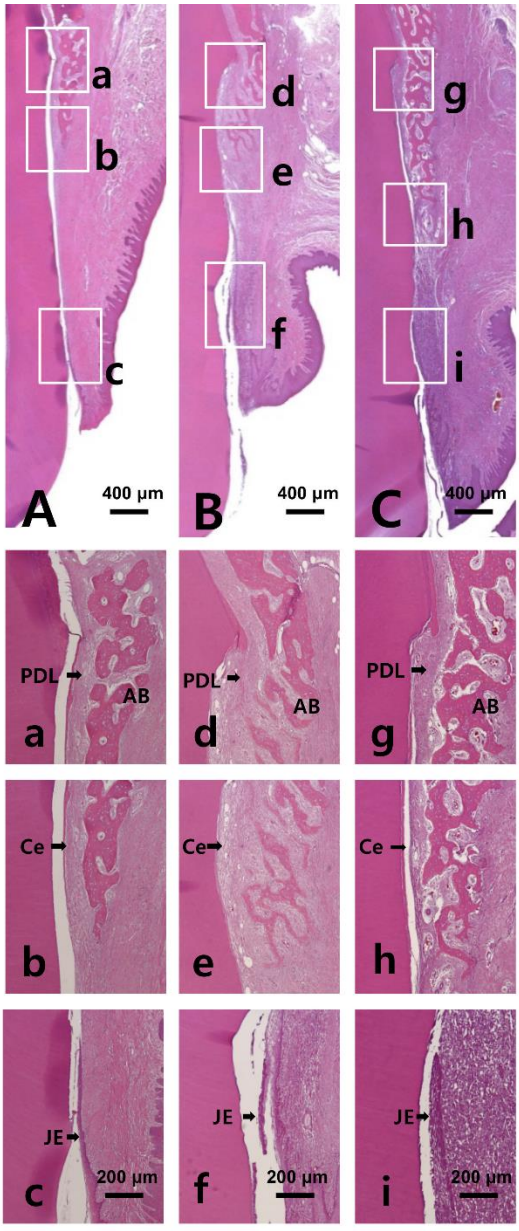


Figure 6

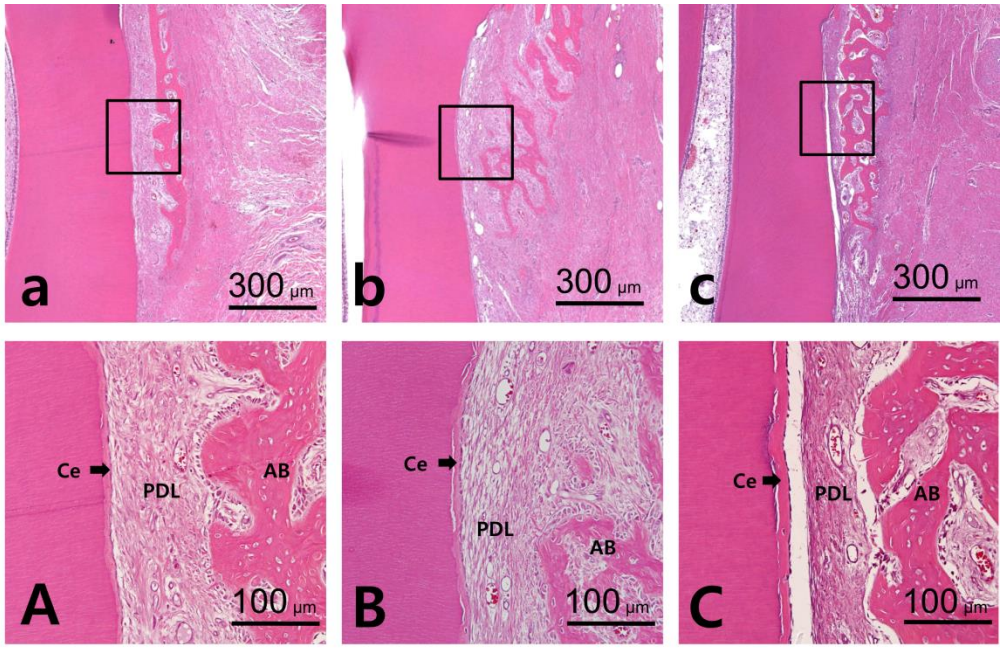
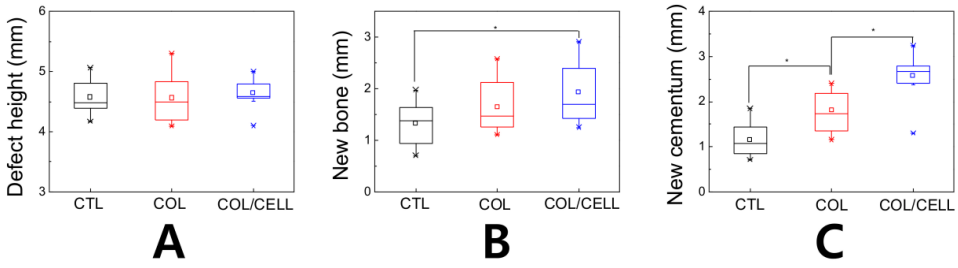


Figure 7



국문요약

성견의 열개결손 모델에서 치주인대 전구세포와 콜라겐매트릭스를 이용한 치주 치유에 대한 연구

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유 승 윤

본 연구의 목적은 성견에서 외과적으로 형성한 열개결손 부위에 치주인대 전구세포와 콜라겐매트릭스를 적용하여 8 후에 치주 치유의 조직학적 특성을 평가하는 것이다.

6 마리의 성견에서 양측의 소구치와 제 1 대구치를 이용하였다. 인위적으로 형성한 규격화된 열개결손을 3 개 소구치의 협측에 형성하였다. 대구치를 이용하여 치주인대 전구세포를 일차배양 하였다. 콜라겐매트릭스는 지지체와 치주인대 전구세포의 전달 매개로 사용되었다. 3 개의 그룹으로 비교하였는데 그룹은 다음과 같다; 콜라겐매트릭스만 이용 (Collagen matrix, COL), 치주인대 전구세포를 적용한 콜라겐매트릭스 (PDL progenitor cells with collagen matrix, COL/CELL), 무처리 (Without any material, CTL). 8

주의 치유기간 후 성견을 희생시켜 조직학적, 조직 계측학적 분석을 위한 시편을 제작하였다.

백악법랑 경계에서 백악질 삭제부의 첨단까지의 열개결손부 높이는 3 개의 그룹에서 CTL 4.57±0.28, COL 4.56±0.41, COL/CELL 4.64±0.27mm 을 나타내어 통계적으로 유의한 차이가 있지 않았다. 상피세포 부착은 CTL 1.41±0.51, COL 0.85±0.29, COL/CELL 0.30±0.41 mm 을 나타내었고, 신생골 생성 높이는 CTL 1.32±0.44, COL 1.65±0.52, COL/CELL 1.93±0.61 mm 을 보였으며, 백악질 생성 길이는 CTL 1.15±0.42, COL 1.81±0.46, COL/CELL 2.57±0.56 mm 으로 모두 그룹간에 유의한 차이를 보였다 ($p<0.05$). 신생골 형성과 백악질 재생에 있어서 COL/CELL 그룹은 CTL 그룹보다 유의하게 높은 수치를 나타내었다. 그러나 생성된 백악질의 두께에 대하여는 그룹간에 차이를 나타내지 않았다.

치주인대 전구세포를 적용한 콜라겐매트릭스는 백악질과 신생골 형성을 포함한 치주조직 재생을 일으키는데 있어서 강화하는 역할을 할 것이다.

핵심되는 말: 열개결손, 치주조직 재생, 치주인대 전구세포, 콜라겐매트릭스