In vivo Evaluation of Decellularized Human Tooth Scaffold for Dental Tissue Regeneration

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In vivo Evaluation of Decellularized Human Tooth Scaffold for Dental Tissue Regeneration

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A Dissertation Thesis
Submitted to the Department of Dentistry
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Dental Science

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December 2020

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감사의 글

박사 학위 논문을 마무리하며, 본 논문이 완성될 수 있도록 물심 양면으로 도와주시고 인도해주신 분들께 깊은 감사의 말씀을 전하고 싶습니다.

우선, 바쁘신 시간에도 제 연구를 이끌어 주시고, 많은 지도를 해 주신 송제선 교수님께 깊은 감사를 드립니다. 치과대학에 이어, 수련 과정 동안 성장할 수 있도록 자상한 지도와 격려를 아끼지 않으신 최병재 교수님과 제가 소아치과의사로 첫 발을 내딛는 시기부터, 귀감의 되고, 열정적인 모습으로 이끌어주신 이제호 교수님, 최형준 교수님, 김성오 교수님, 강정민 교수님께도 감사를 전합니다.

또한 논문에 대해 조언을 해주시고, 아낌없이 지도해주신 정한성 교수님, 신유석 교수님 모두 감사 드립니다. 아울러 많이 부족한 저의 수많은 질문과 요구사항들, 실험 설계 및 진행에 많은 도움을 준 전미정 교수님께도 감사를 전합니다.

지금까지 제가 발전할 수 있도록 멀리서 응원해주시고, 고생하신 부모님과, 비록 자주 보지는 못하지만 같은 길을 앞에서 이끌어 주고 있는 형님과 가족들이 큰 힘이 되었습니다. 그리고 제가 가고 싶은 길에 대해 응원을 아끼지 않으신 장인어른과 장모님, 힘들지만 묵묵히 곁에서 힘이 되어준 아내 서연에게 감사하며, 부족한 저를 도와주신 모든 분들과 함께 존경과 사랑을 담아 이 작은 결실을 나누고 싶습니다.

아직 많이 부족한 제가 가야할 길이 멀기에, 앞으로도 많은 지도와 격려 부탁드리며, 도움을 주신 많은 분들께 진심으로 감사드립니다.

2020년 12월

김익환 드림

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(Directed by Professor Je Seon Song, D.D.S.,M.S.,Ph.D.)

Regeneration of periodontal ligaments and pulp is important in the recovery of damaged teeth. Conventional treatment methods proceed with the removal and replacement of damaged tissue, which leads to loss of tooth vitality. Loss of vitality of the teeth can lead to poor dental prognosis, and especially in the case of immature permanent teeth, the development of teeth can be stopped. Research related to the regeneration of dental pulp and periodontal ligament tissue is being actively conducted in order to restore the vitality of teeth and maintain biological functions, and the scaffold is one of the important factors in terms of tissue regeneration. Therefore, this study is to confirm the

regeneration of periodontal ligament and pulp tissue using a decellularized human tooth as a scaffold.

A decellularized tooth scaffold was fabricated using the combination method of sodium dodecyl sulfate and Triton X-100, an effective decellularization method identified in previously studies. Dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs) obtained from human permanent teeth were inoculated onto decellularized scaffolds and cultured, and then transplanted into immunosuppressed mouse to induce tissue regeneration for 9 weeks. After that, the transplants were separated and analyzed for histological analysis, immunochemical staining, and quantitative real-time polymerase chain reaction (qPCR) on the regenerated tissue.

In the case of PDLSCs, it was observed that the inoculated cells penetrated to the inside of the periodontal ligament extracellular matrix of the decellularized scaffold, survived and differentiated, and high expression of cementum-derived protein 23 (CP23), collagen type I (Col I), collagen type XII (Col XII), osteocalcin (OC), and alkaline phosphatase (ALP) was observed. As a result, the possibility of regeneration of the cementum/periodontal ligament complex could be expected.

DPSCs also survived and differentiated in the extracellular matrix of the pulp tissue of the decellularized scaffold, and high expression of dentin sialophosphoprotein (DSPP), dentin matrix acidic phosphoprotein 1 (DMP1), nestin (NES), Col I, ALP, cluster of differentiation 31 (CD31), and cluster of differentiation 34 (CD34) was observed. In addition, the expression of genes related to revascularization and the generation of newly

formed hard tissue showed the possibility of pulp tissue regeneration.

Through this study, it was possible to confirm the possibility of using a decellularized human tooth as a scaffold for regeneration of periodontal ligament and pulp tissue using PDLSCs and DPSCs, and this could be applied to novel treatment methods and research on the regeneration of dental tissue.

Keywords: Decellularized tooth scaffold, periodontal ligament stem cell, dental pulp stem cell, tissue regeneration

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I. Introduction

The tooth is composed of enamel, dentin, cementum, pulp, and periodontal ligament (PDL). Although teeth are physiologically and structurally complex, dental pulp and PDL are also very vulnerable to stimulations, such as caries, infections, and trauma (Lambrichts, et al., 2017). In this case, the conventional treatment method is to remove damaged tissue or replace it with dental material, which leads to loss of biological function. Also, immature permanent teeth may stop developing. The prognosis of teeth that lose these biological functions or remain in an immature state can be poor in many ways (Chueh, et al., 2009;

Cvek, 1992; Kling, et al., 1986; Nene and Bendgude, 2018). Research to replace conventional treatments through tooth regeneration is continually being conducted. In particular, pulp and PDL regeneration studies are the most active (Eramo, et al., 2018; Hu, et al., 2018; Isaka, et al., 2001; Nakashima, et al., 2017; Yen and Sharpe, 2006).

The elements of tissue engineering that induce regeneration are scaffold, cell, and growth factor (Bianco and Robey, 2001; Hubbell, 1995). Stem cells (SCs) refer to a type of cell that have the potential to proliferate, self-renew, and differentiate into a variety of functional cells in a certain condition (Weissman, et al., 2001). The dental stem cells (DSCs) are post-natal stem cell populations that have mesenchymal stem cell-like qualities, including the capacity for self-renewal and multi-lineage differentiation potential (Chalisserry, et al., 2017). Also, the DSCs play an important role in tooth repair (Sharpe, 2016). In oral tissue, stem cells can be collected from craniofacial bone, dental follicle, tooth germ, dental pulp, PDL, apical papilla, oral mucosa, gingiva, and periosteum (Egusa, et al., 2012). Various DSCs have therapeutic potential, and there are active studies to achieve regeneration of tooth tissues using them (Chalisserry, et al., 2017).

Scaffolds provide cell adhesion and enable cell proliferation, mimicking the microenvironment observed in natural tissues and organs (Bohl, et al., 1998). Therefore, the role of scaffold in tissue regeneration using stem cells is very important. Research related to tissue regeneration is actively progressing, but repairs are still more likely than true tissue regeneration. Various types of scaffolds are being studied for better results (Bružauskaitė, et al., 2016; Srinivasan, et al., 2012). In fact, products that can be used in

dental pulp tissue engineering are commercially available (Galler, et al., 2012). As part of these studies, decellularized tissue has focused in regeneration engineering, and the use of decellularized human tooth scaffolds has also been studied in dental regeneration (Son, et al., 2019; Song, et al., 2017).

The periodontium, which means the tissue around the teeth, plays a major role in maintaining teeth, and PDL is directly related to the vitality of the teeth (Narayanan, 1983). Like Pulp, if PDL is damaged, it has a great adverse effect on the prognosis of teeth, and regeneration of PDL is also very difficult. Studies related to scaffold, cell sheets and periodontal ligament stem cells(PDLSCs) for periodontal regeneration are also being actively conducted (Song, et al., 2015; Zhu and Liang, 2015).

Conventional root canal treatments that replace necrotic tissue with bio-inert materials lose vitality in the teeth, making them susceptible to secondary infections and post-operative fractures (Cordeiro, et al., 2008; Qu, et al., 2014). In order to recover the vitality of teeth and maintain development in immature permanent teeth, pulp regeneration is important, and regenerative endodontic procedures (REPs) can overcome such limitations (Albuquerque, et al., 2014; Diogenes, et al., 2016). However, these REPs formed dental pulp-like tissue, but successful regeneration of vascularized pulp tissue was observed with in situ transplantation of human dental pulp stem cells (DPSCs) (Iohara, et al., 2011; Iohara, et al., 2013). Compared with the cell homing-based method, direct transplantation of stem cells showed higher capillary density in newly formed tissues (Takeuchi, et al., 2015).

This study tested the hypothesis that decellularized human tooth scaffold can retain their

extracellular matrix integrity and support recellularization and promote differentiation with stem cells. The purpose of this study is to show that decellularized human tooth scaffold can promote the differentiation using DPSCs and PDLSCs *in vivo*.

II. Materials and Methods

1. Tooth Sample Preparation and Cell Culture

Samples were prepared as described in previous study (Song, et al., 2017). Briefly, the second premolars, free of caries and restorations were randomly collected from patients of 17 to 25y of age under approved guidelines set by the Institutional Review Board of the Dental Hospital, Yonsei University (approval no. #IRB 2-2016-0030). Teeth were washed with phosphate buffered saline (PBS; Invitrogen, Carlsbad, CA, USA) and submerged in 0.5% Chloramine T (Sigma-Aldrich, St. Louis, MO, USA) for 2h at 4℃, followed by washes in cold running water. The pulp tissues were removed with barbed broach (Mani, Utsunomiya Tochi-ken, Japan) for decellularized human periodontal ligament [dHPDL], and the PDL tissues were removed with dental currettes for decellularized human dental pulp [dHDP]. The tooth specimens were prepared using an IsoMet 1000 precision saw (Buehler Ltd., Evanston, IL, USA) as previously described (Cordeiro, et al., 2008) in two types; semi-cylinder-shaped for dHPDL and doughnut-shaped for dHDP (Figure. 1). Samples were collected in cold PBS and immediately subjected to the decellularization procedures.

A previously characterized PDLSCs and DPSCs (Cha, et al., 2015; Son, et al., 2019; Song, et al., 2012; Song, et al., 2017) from three to six passages were used in recellularization experiments in a basal cell culture medium comprising alpha-minimum essential medium

(a-MEM; Invitrogen) containing 10% fetal bovine serum (FBS; Invitrogen), 1% L-glutamine/penicillin/streptomycin solution (Invitrogen), and 0.2% amphotericin B solution (Invitrogen) at 37℃ in 5% CO_2. Briefly, PDL tissues from the permanent teeth were obtained carefully using sterile curettes from the middle-third and pulp tissues from the permanent teeth were extirpated using a barbed broach, washed with PBS (Invitrogen), and subjected to primary culture using outgrowth method. PDL or pulp tissues were placed onto a 60-mm culture dish (BD Falcon, Lincoln Park, NJ, USA), and covered with a cover glass to allow the cells to grow out. The cells were cultured in the culture medium described above at 37 ℃ in 5% CO_2.

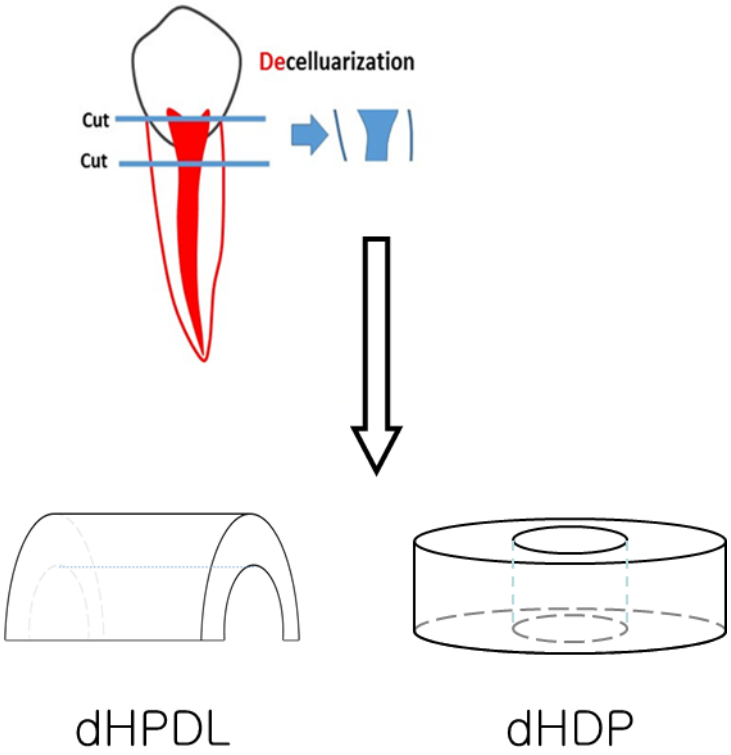

Figure 1. Prepare the decellularized tooth scaffold. The tooth specimens were prepared in two types; semi-cylinder-shaped for decellularized human periodontal ligament (dHPDL) and doughnut-shaped for decellularized human dental pulp (dHDP).

2. Decellularization and Recellularization

PDLSCs and DPSCs were cultured from based on a previous study (Son, et al., 2019; Song, et al., 2017), 1% Triton X-100 (Bio Basic Inc., Markharn, ON, Canada) and 1% sodium dodecyl sulfate (SDS; Tech & Innovation, Gangwon, Korea) were used for decellularization protocol. Briefly, tooth slices were incubated in 1% Triton X-100 for 24hours, and 1% SDS for 24 hours. This cycle were repeated by 3 times. All treatment steps were performed on tooth slices at room temperature with constant gentle agitation of the samples in an orbital shaker (SH30, Fine PCR, Gyeonggi, Korea), in the presence of protease inhibitor cocktail (EMD Millipore, Darmstadt, Germany). At the end of each protocol, samples were rinsed with 10% ethylenediaminetetraacetic acid (EDTA, pH7.4; Fisher Scientific Co., Houston, TX, USA) for 5 min, followed by 3 rinses of 10 min each with PBS (Invitrogen).

For recellularization of the decellularized tooth slices (dHPDL and dHDP), PDLSCs and DPSCs at a density of $1x10^7$ cells/mL in rat tail collagen I (Corning Inc., Corning, NY, USA) were pipetted directly onto dHPDL and dHDP respectively in 12-well culture plates (Corning Inc.). After 30mins, 1ml of basal culture media was applied to each well and changed every 3 days. Cells were cultured at 37℃ in 5% CO_2 for either 2 weeks.

3. *In vivo* Transplantation

These procedures were performed in accordance with protocol approved by the Institutional Animal Care and Use Committee of Yonsei University (#2016-0229). Samples were prepared according to the recellularization experiments. dHPDL and dHDP without recellularization were used for control. Samples were subcutaneously transplanted into the dorsal surface of 5-week male immunocompromised mice (BALB/c-nu, SLC, Shizuoka, Japan) (Figure. 2). Four pockets were made per each mouse (n=20), and two different types of transplants were individually inserted into the pocket; recellularized PDLSCs or recellularized DPSCs and dHPDL/dHDP without recellularization, 20 pockets were used respectively. All transplants were retrieved after 9 weeks posttransplantation; of these 20, 6 from each group were subjected to histological and immunohistological analysis, and the remaining 14 transplants from each group were used for quantitative real-time polymerase chain reaction (qPCR).

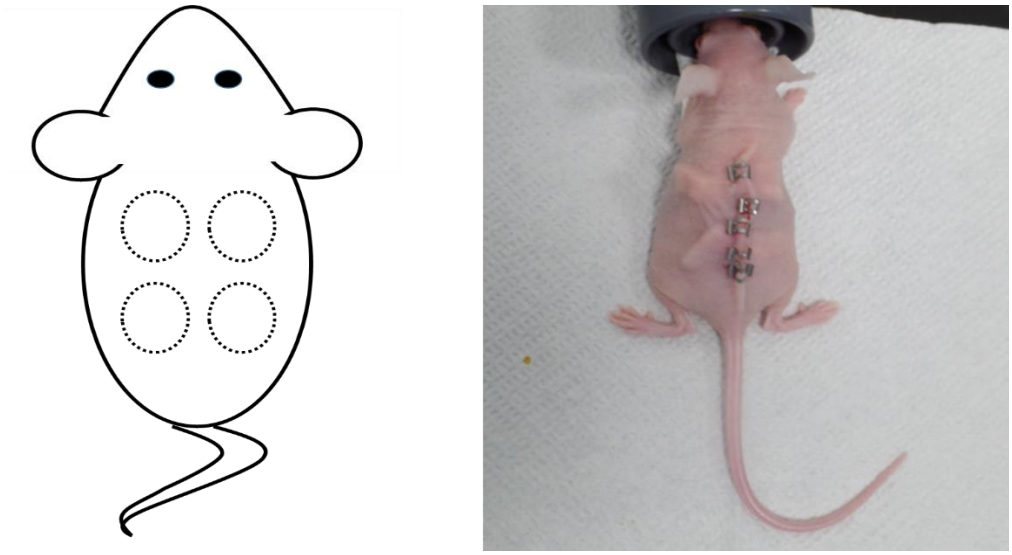

Figure 2. *In vivo* transplantation scheme. Samples were subcutaneously transplanted into the dorsal surface of mice. Four pockets were made per each mouse and two different types of transplants were individually inserted into the pocket; recellularized periodontal ligament stem cells (PDLSCs) and control (dHPDL without recellularization) or recellularized dental pulp stem cells (DPSCs) and control (dHDP without recellularization).

4. Histology and Immunohistochemistry (IHC)

For histological staining, transplants were fixed with 10% formalin for an hour, decalcified with erhylenediaminetetraacetic acid (EDTA, pH7.4; Fisher Scientific Co., Houston, TX, USA) for 9 wks at room temperature, embedded in paraffin sectioned at a thickness of 4 μm, and stained with hematoxylin and eosin (HE) and Masson's trichrome (MT) staining.

For IHC staining, the sections were deparaffinized in xylene, rehydrated, and rinsed with distilled water. For antigen retrieval, protease K (Dako, Carpinteria, CA, USA) was used for cementum-derived protein 23 (CP23; Abcam, Cambridge, UK), osteocalcin (OC; Merck millipore, Darmstadt, Germany) staining, and vascular endothelial growth factor (VEGF; Abcam), 10 mM citrate buffer (pH6.0) was used for cluster of differentiation 34 (CD34; Abcam), human nuclei (HN; Merck Millipore) staining, while no such treatment was performed for collagen type XII (ColXII; Abcam) and dentin sialoprotein (DSP; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were immersed in 3% hydrogen peroxide for 10 min to inactivate endogenous peroxidase activity and then incubated with primary antibody overnight. The information of primary antibodies was given in table 1. After incubation, EnVision + System-HRP Labeled Polymer Anti-mouse (K4001, Dako; ready to use) and EnVision + System-HRP Labeled Polymer Anti-rabbit (K4003, Dako; ready to use) was applied for 20 min or Vectastain Elite ABC Kit (PK-6105, Vector Laboratories, Burlingame, CA, USA; goat IgG, diluted 1:200) was applied for 30 minutes.

Color development was performed using 3,3'-diaminobenzidine substrate (Dako) and counterstained with Gill's hematoxylin solution (Merck millipore). Negative control sections were treated in the same manner but without primary antibodies.

Table 1. Primary antibodies for immunohistochemistry.

Antibodies	Catalog number	Host species	Dilution factor
CD34	Ab110643	Rabbit	1:100
Col XII	Sc-68862	Rabbit	1:2000
CP23	Sc-164031	Goat	1:500
DSP	Sc-33586	Rabbit	1:500
HN	MAB1281	Mouse	1:100
OC	AB10911	Rabbit	1:8000
VEGF	Ab183100	Goat	1:100

Abbreviations: CD34, cluster of differentiation 34; Col XII, collagen type XII; CP23, cementum-derived protein 23; DSP, dentin sialoprotein; HN, human nuclei; OC, osteocalcin; VEGF, vascular endothelial growth factor.

5. Gene Expression Analysis by quantitative RT-PCR (qRT-PCR)

The relative gene expressions in the transplants were confirmed by qRT-PCR. In brief, total RNA was extracted from the transplants by using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The integrity and concentration of extracted RNA was measured using a spectrophotometer (NanoDrop ND-2000, ThermoScientific, Waltham, MA, USA). cDNA was synthesized from RNA (500ng) using a Maxime RT PreMix kit (oligo d(T)15 primer; Intron Biotechnology, Seoul, Korea) according to the manufacturer's instructions. A qRT-PCR assay was performed with SYBR Premix EX Taq (Takara Bio, Otsu, Japan) and an ABI 7300 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA), also according to the manufacturer's instructions. The information of the primers are given in table 2. The expression level of each gene was normalized to that of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the relative expression levels of genes was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The expression level of each gene in the transplants was calculated relative to its expression level in the cell-free dHPDL or dHDP transplants (control).

Table 2. Quantitative real-time polymerase chain reaction (qPCR) forward and reverse primer sequences. The annealing procedures were performed at 60ºC for all primers.

Gene	Forward Primer sequence (5'–3')	Reverse Primer sequence (5'–3')
ALP	GGACCATTCCCACGTCTTCAC	CCTTGTAGCCAGGCCCATTG
CD31	CCCATTGTTCCCGGTTTCCA	AGTTAGTTCTGCCTTCGGGC
CD34	CGCTGCCTTGCCAAGACTAA	CCTAGAGAGACGCACCGAGT
Col I	CGATGGCTGCACGAGTCACAC	CAGGTTGGGATGGAGGGAGTTTAC
Col XII	CGGACAGAGCCTTACGTGCC	CTGCCCGGGTCCGTGG
CP23	AACACATCGGCTGAGAACCTCAC	GGATACCCACCTCTGCCTTGAC
DMP1	GATCAGCATCCTGCTCATGTT	AGCCAAATGACCCTTCCATTC
DSPP	GGGATGTTGGCGATGCA	CCAGCTACTTGAGGTCCATCTTC
NES	GCCCTGACCACTCCAGTTTA	GGAGTCCTGGATTTCCTTCC
OC	CAAAGGTGCAGCCTTTGTGTC	TCACAGTCCGGATTGAGCTCA
POSTN	CACAACCTGGAGACTGGAC	TGTCTGCTGGATAGAGGAG
GAPDH	TCCTGCACCACCAACTGCTT	TGGCAGTGATGGCATGGAC

Abbreviations: *ALP*, gene encoding alkaline phosphatase; *CD31*, gene encoding cluster of differentiation 31; *CD34*, gene encoding cluster of differentiation 34; *Col I*, gene encoding collagen type I; *Col XII*, gene encoding collagen type XII; *CP23*, gene encoding cementum-derived protein 23; *DMP1*, gene encoding dentin matrix acidic phosphoprotein 1; *DSPP*, gene encoding dentin sialophosphoprotein; *NES*, gene encoding nestin; *OC*, gene encoding osteocalcin; *POSTN*, gene encoding periostin; *GAPDH*, gene encoding glyceraldehyde-3-phosphate dehydrogenase.

6. Statistical analysis

All experiments were performed at least in triplicate. Statistical analysis was performed with SPSS software (version 25.0, SPSS, Chicago, IL, USA). The normality of the data was evaluated using the Shapiro-Wilk test ($p<0.05$). The Mann-Whitney U test ($p<0.05$) was performed for all experiments using SPSS software (SPSS, USA).

III. Results

1. Histological and immunohistochemical analysis of PDLSCs recellularization after *in vivo* transplantation

PDLSCs were repopulated on dHPDL, and viability was observed after 9 weeks of transplantation in mouse pockets. Unlike the control group in which cells were not inoculated, in the PDLSCs group, repopulation in the dHPDL ECM was observed in HE and MT staining (Figure. 3A-B,D-E). In addition, HN staining confirmed that the cells were of human origin (Figure. 3C,F). Through immunohistochemical staining, anti-human Col XII, CP23, and OC were identified. Unlike the control group, it was found that the PDLSCs group was stained (Figure. 4).

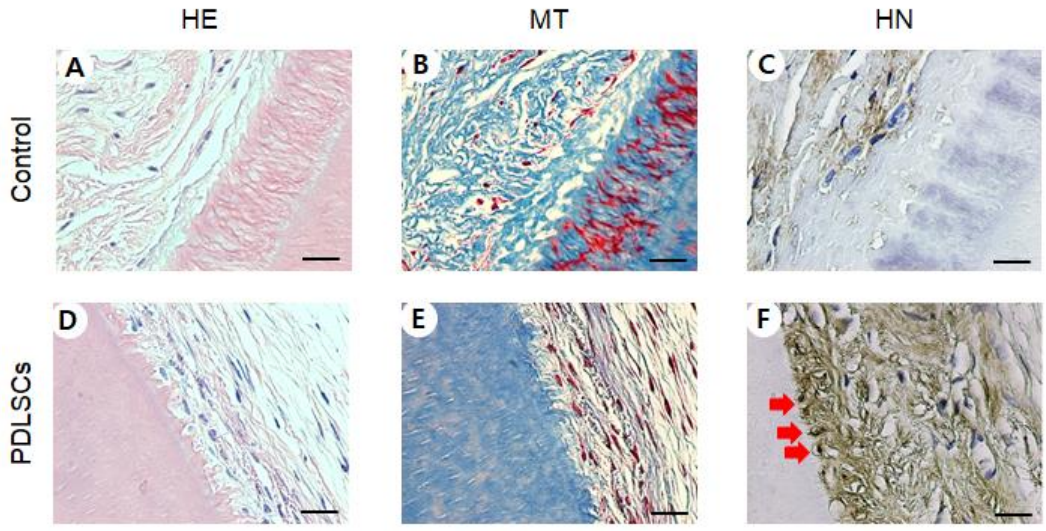

Figure 3. Histological characteristics of dHPDL transplants recellularization of PDLSCs. Hematoxylin and eosin (HE) staining (A,D), Masson's trichrome (MT) staining (B,E), and human nuclei (HN) staining (C,F). HE, MT, and HN staining showed recellularized cells are present in dHPDL transplants. Recellularized cells are indicated by red arrows. Scale bars: 20 μm.

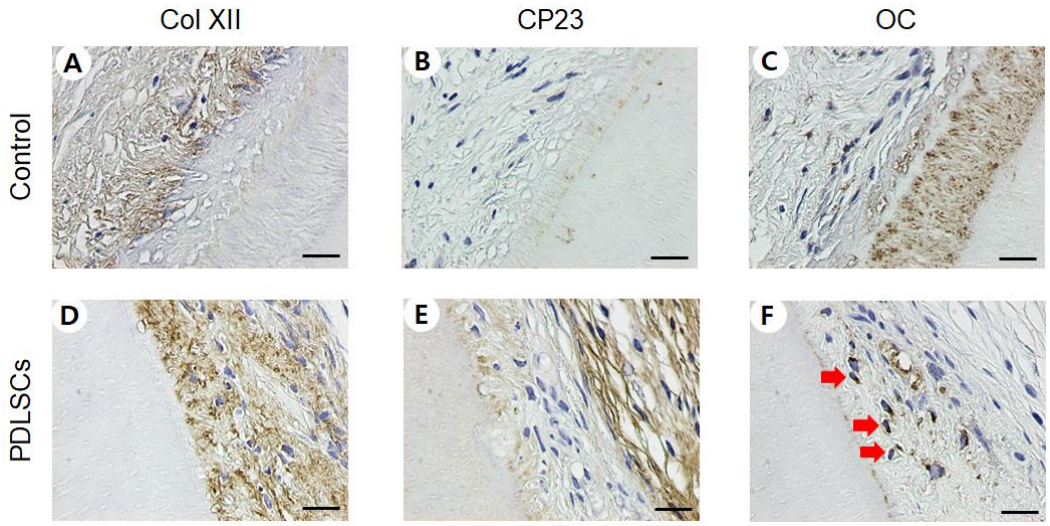

Figure 4. Immunohistochemical staining of dHPDL transplants recellularization of PDLSCs. dHPDL transplants immunostained with anti-human collagen type XII (Col XII; A,D), anti-human cementum-derived protein 23 (CP23; B,E), and anti-human osteocalcin (OC; C,F) antibodies. The red arrows indicate examples of positively immunostained cells. Scale bars: 20 μm.

2. Gene expression of PDLSCs recellularization after *in vivo* transplantation

qPCR was performed to assess the gene expression of markers by PDLSCs recellularization. There was significant difference in the expression of *CP23*, *Col I*, *Col XII*, *OC*, and alkaline phosphatase (*ALP*) on PDLSCs recellularization group. Otherwise, there was no significant difference in the expression of periostin (*POSTN*) (Figure. 5).

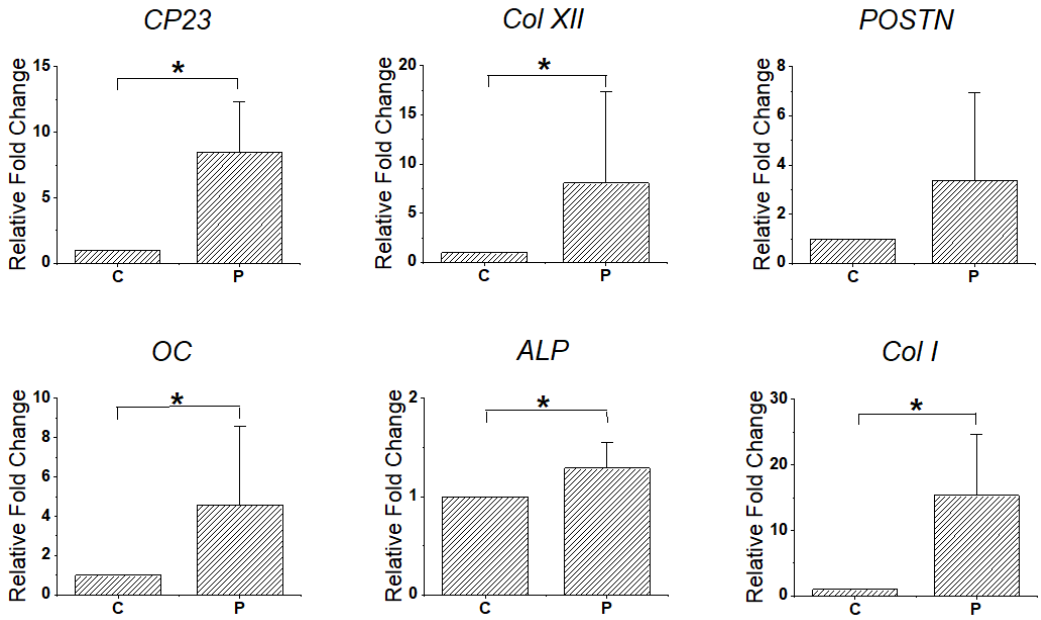

Figure 5. Relative expression levels of the genes encoding *CP23*, *Col XII*, *POSTN*, *OC*, *ALP*, and *Col I* in dHPDL transplants. Data are mean and standard deviation values. The expressions of *CP23*, *Col XII*, *OC*, *ALP*, and *Col I* differed significantly between the two groups (*Mann-Whitney U test, $p<0.05$). The expression of *POSTN* did not differ significantly between the two groups (Mann-Whitney U test; $p>0.05$). C, Control group; P, PDLSCs recellularization group. Abbreviations: *CP23*, *cementum-derived protein 23*; *Col XII*, *collagen type XII*; *POSTN*, *periostin*; *OC*, *osteocalcin*; *ALP*, *alkaline phosphatase*; *Col I*, *collagen type I*.

3. Histological and immunohistochemical analysis of DPSCs recellularization after *in vivo* transplantation

DPSCs were repopulated in dHDP, and the results were confirmed after 9 weeks in the same method as in PDL. In the DPSCs group, the repopulated cells could be observed through HE and MT staining, and similarly, the human origin was confirmed through HN staining (Figure. 6). Un like the control group, in the DPSCs group, the newly formed hard tissues were observed on HE staining (Figure. 6A,D). In the DPSCs group, the mineralized matrix presented a poor level of organization, featuring intermingled alignment of matrix and embedded cells with a large cytoplasmic space and large nuclei (Figure. 6D). Through immunohistochemical staining, anti-human DSP, OC, VEGF, CD34 were identified. Unlike the control group, it was found that the DPSCs group was stained (Figure. 7).

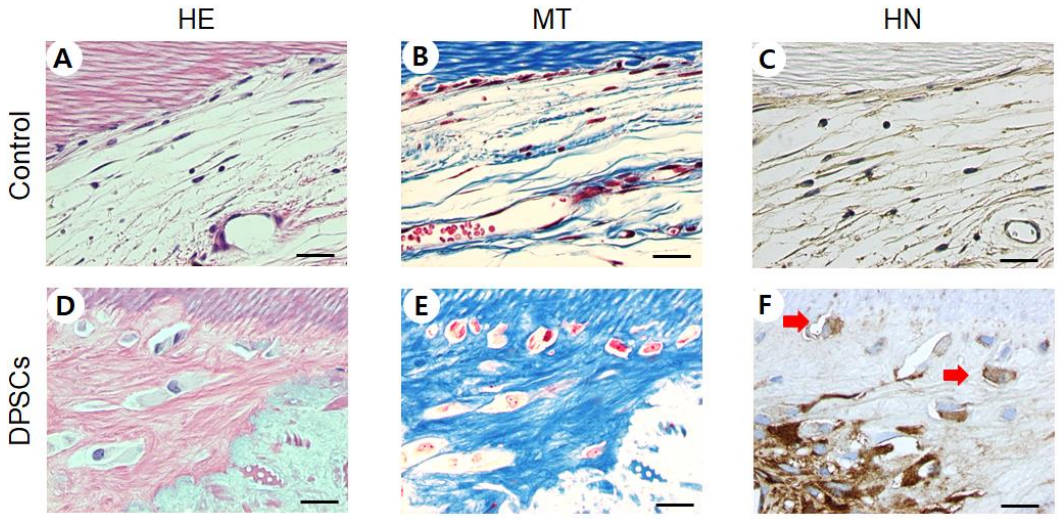

Figure 6. Histological characteristics of dHDP transplants recellularization of DPSCs. HE staining (A,D), MT staining (B,E), and HN staining (C,F). HE, MT, and HN staining showed recellularized cells are present in dHDP transplants. Recellularized cells are indicated by red arrows. Scale bars: 20 μm.

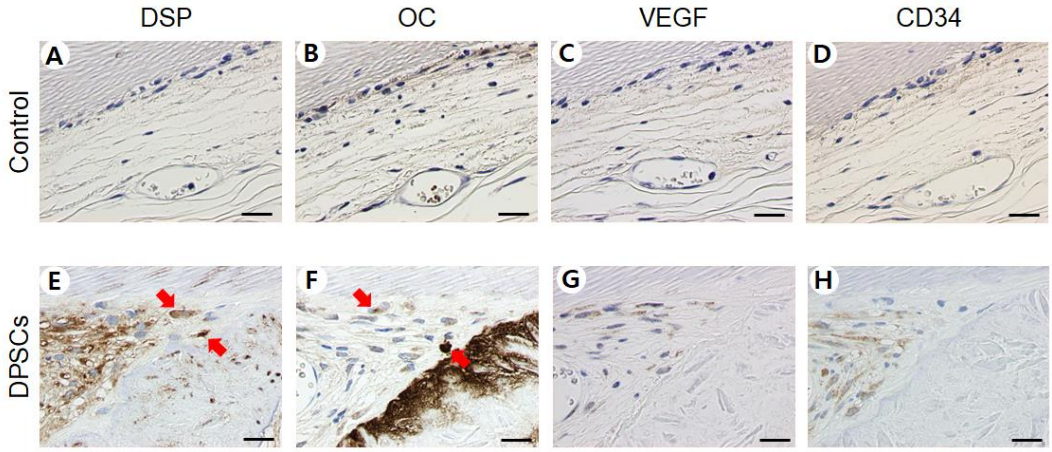

Figure 7. Immunohistochemical staining of dHDP transplants recellularization of DPSCs. dHDP transplants immunostained with anti-human dentin sialoprotein (DSP; A,E), anti-human OC (B,F), anti-human vascular endothelial growth factor (VEGF; C,G), and anti-human cluster of differentiation 34 (CD34; D,H) antibodies. The red arrows indicate examples of positively immunostained cells. Scale bars: 20 μm.

4. Gene expression of DPSCs recellularization after *in vivo* transplantation

qPCR was also performed to confirm the expression of the relevant marker in the DPSC recelluarization. In the DPSC recellularization group, the expression of not only genes related to DPSC but also genes related to hard tissue formation was confirmed, and expression of genes related to blood vessels and nerves was also confirmed. There was a significant difference in the expression of all identified genes except *OC* (Figure. 8).

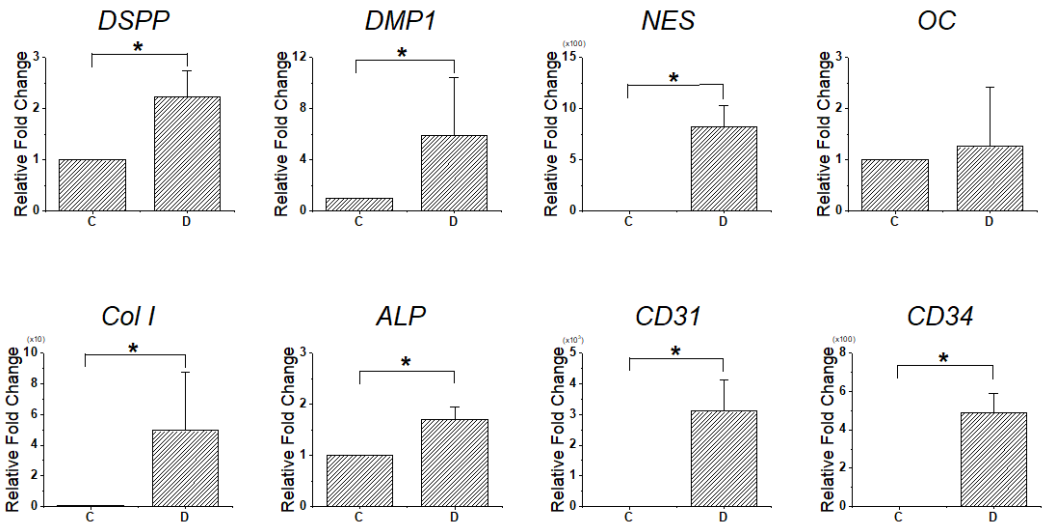

Figure 8. Relative expression levels of the genes encoding *DSPP*, *DMP1*, *NES*, *OC*, *Col I*, *ALP*, *CD31*, and *CD34* in dHDP transplants. Data are mean and standard deviation values. The expressions of *Col I*, *DMP1*, *NES*, *Col I*, *ALP*, *CD31*, and *CD34* differed significantly between the two groups (*Mann-Whitney U test : $p<0.05$). The expression of *OC* did not differed significantly between the two groups (Mann-Whitney U test; $p>0.05$). C, Control group; D, DPSC recellularization group. Abbreviations: *DSPP*, *dentin sialophosphoprotein*; *DMP1*, *dentin matrix acidic phosphoprotein 1*; *NES*, *nestin*; *CD31*, *cluster of differentiation 31*; *CD34*, *cluster of differentiation 34*.

IV. Discussion

In the present study, I evaluated the differentiation of stem cells inoculated in the decellularized human dental tooth scaffold. In tissue regeneration, scaffold is a very important factor along with cell and growth factors. Proper scaffolds provide structural support and play an important role in cell function and differentiation of new tissue formation (Chen, et al., 2002; Hutmacher, 2001). Actually, many decellularized tissues such as skin, blood vessel, bone, and cartilage are used as graft materials for patients (Gilbert, et al., 2006; Porzionato, et al., 2018). Appropriately decellularized tissues preserve not only the ECM integrity, bioactivity, and spatial structure but also the vascular, lymphatic, and nervous network (Badylak, et al., 2012).

However, in the decellularized scaffold, all cellular components must be removed as they can cause immune rejection (Keane, et al., 2012). Scaffold decellularization method and repopulation results are affected by various factors such as tissue type and composition, thickness, density and cellularity of the tissue (Lu, et al., 2012). In previous studies, an optimized decellularization protocol was established through various methods. Additionally, an effective method of removing residual DNA, HLA-A, and cellular β-actin using human dental tooth was evaluated (Son, et al., 2019; Song, et al., 2017).

The recellularization potential of decellularized pulp and PDL scaffold was evaluated by real-time PCR and IHC staining. When stem cells were repopulated in both Pulp and PDL

scaffolds, distinctive results were observed in the formation of new tissues compared to the control group not inoculated with cells.

Similar to the results of *in vitro* experiments previously studied (Song, et al., 2017), PDLSCs were well engrafted around the site near the cementum, and it was confirmed that PDLSCs retain their unique characteristics through high expression of PDLSCs-specific markers CP23 and POSTN (e.g.,CP23, identified as a cementoblast marker and regulator of the bio-mineralization of cementum (Alvarez-Pérez, et al., 2006; Villarreal-Ramírez, et al., 2009), and POSTN, identified as a PDL marker and found mainly in cells from mesenchymal lineage such as osteoblast, periodontal ligament, and periosteum (Bolton, et al., 2009; Romanos, et al., 2014)) Particularly, as a result of using the semi-cylindrical dHPDL, it was confirmed that PDLSCs migrated deeply to the part close to the cementum by passing through the collagen network of dHPDL (Fig. 3 D,E,F and Fig. 4 D,E,F). In particular, it was confirmed that the expression of Col XII, a marker of mature PDL, was increased (Karimbux and Nishimura, 1995), indicating that PDLSCs differentiated into more mature PDL. In addition, it was difficult to confirm the newly formed hard tissue through HE staining, but the expression of OC and ALP involved in mineralization of cementum and bone was confirmed (McKee, et al., 1996; Stucki, et al., 2001). Therefore, recellularization of PDLSCs may move to near the cementum of dHPDL, affect the generation of mature PDL, and differentiation of hard tissues *in vivo*.

DPSCs showed different characteristics from PDLSCs. In the DPSCs recellularization group, the formation of newly formed hard tissue was confirmed through HE staining. The

newly created hard tissue did not have a dentin-like structure, but the expression of hard tissue-related markers such as DSPP, DMP1, ALP OC, and Col I was confirmed (Batouli, et al., 2003; Conde, et al., 2015; Stucki, et al., 2001; Su, et al., 2014; Thomson, et al., 2003). In addition, the expression of nestin related to nerve and CD31, CD34 related to angiogenesis were confirmed, showing that the formation of hard tissue and nerve and blood vessel generation may be possible. Since the remodeling of blood vessels plays a role in the supply of essential elements necessary for tissue regeneration, vascularization is one of the important factors. In particular, angiogenesis was also confirmed by the expression of VEGF in IHC and the expression of CD31 and CD34 in qRT-PCR. VEGF is a marker for angiogenesis, and it has been reported that angiogenesis accompanies the process of osteogenesis. CD31 is the most used marker for evaluating angiogenesis and CD34 is present in hematopoietic progenitor cells and endothelial cells and has been studied as a marker for vascular tumors (Stross, et al., 1989; Traweek, et al., 1991). And it has been reported that anti-CD34 antibody is a highly sensitive marker for endothelial cell differentiation, which stains neoplastic endothelium a deeper shade than normal endothelium (Kuzu, et al., 1992). The generation of blood vessels facilitates the supply of nutrients, oxygen, minerals essential for mineralization, and bone-forming progenitor. In particular, osteogenic factors such as BMP2 have been reported to promote osteoblast differentiation and mineralization by being secreted from blood vessels (Hu and Olsen, 2016; Matsubara, et al., 2012). Thus, mature osteoblasts generate mature angiogenic factors such as VEGF, which can also promote angiogenesis. These results can be expected to

proceed positively in the tissue regeneration process. Furthermore, when endothelial cells are co-seeded with stem cells in the scaffold, the roles of factors that can positively affect osteogenesis and vascularization can be expected (Liu, et al., 2017).

Teeth are complex organs made up of various cells and structures. When these are damaged, regeneration is not easy. In particular, soft tissues such as PDL and pulp play an important role in physical, formative, nutritional, and sensory functions, and are related to tooth vitality (Beertsen, et al., 1997; Van Hassel, 1971).

The PDL is the soft connective tissue that connects the root of the tooth and the inner wall of the alveolar socket (Beertsen, et al., 1997). PDL can be damaged by local causative factors such as plaque, dental calculus and traumatic occlusion, and can be destroyed by systemic factors or external factors such as smoking. In addition, PDL can be damaged by external force, especially when avulsion rather than luxation. Such damage to PDL can cause the tooth to fall out in the same way as the root resorption, so recovery of damaged PDL is very important for tooth recovery (Andreasen, 2012; Yen and Yelick, 2011).

The pulp can be necrotized due to caries, trauma, and anatomic variations such as dens evaginatus and dens invaginatus (Diogenes, et al., 2013). The damaged pulp was removed and replaced with dental material, which means the loss of vitality of the tooth. These results are particularly fatal in immature permanent teeth, and stop the development of teeth, resulting in poor clinical prognosis. REPs have made a lot of progress in recent years, and are positioned as an alternative treatment that can preserve tooth vitality in immature permanent teeth, which enables continuous tooth growth and development (Diogenes, et

al., 2016). But despite the clinical success of these procedures, they appear to promote a guided endodontic repair process through cell homing rather than a true regeneration of pulp-like tissue (Kim, et al., 2010).

Dental tissue therapies are transitioning from traditional dentistry to biological based treatment, and the success rates of treatment are gradually increasing (Schmalz and Smith, 2014). The goal of traditional dental treatment is to restore the function and morphologic integrity of the damaged tooth. However, the goal of recent changes in dental treatment is maintenance of tooth vitality.

The decellularized human tooth scaffold used in this study maintained their structure, and collagen remained in the ECM in both pulp and PDL. Each Scaffold has recellularization potential when cultured *in vivo* after stem cell inoculation, and when DPSC or PDLSC, which are cells of different characteristics, were applied to the scaffold, gene expression related to pulp and PDL regeneration was induced. Mesenchymal stem cells applied to decellularized human tooth scaffolds have the potential to open a novel approach to pulp and PDL regeneration through proliferation and differentiation. In addition, when two cells are recellularized in the same decellularized tooth scaffold, simultaneous regeneration of pulp and PDL can be expected using one scaffold.

V. Conclusion

In this study, when using DPSCs and PDLSCs for pulp and PDL regeneration, it was confirmed that decellularized human teeth can be used as a scaffold. DPSCs and PDLSCs inoculated into decellularized human tooth scaffolds and cultured in an *in vivo* environment showed successful differentiation into corresponding tissues.

VI. References

Albuquerque M, Valera M, Nakashima M, Nör J, Bottino M: Tissue-engineering-based strategies for regenerative endodontics. *Journal of dental research* 93(12): 1222-1231, 2014.

Alvarez-Pérez MA, Narayanan S, Zeichner-David M, Rodríguez Carmona B, Arzate H: Molecular cloning, expression and immunolocalization of a novel human cementum-derived protein (CP-23). *Bone* 38(3): 409-419, 2006.

Andreasen JO: Pulp and periodontal tissue repair-regeneration or tissue metaplasia after dental trauma. A review. *Dental traumatology* 28(1): 19-24, 2012.

Badylak SF, Weiss DJ, Caplan A, Macchiarini P: RETRACTED: Engineered whole organs and complex tissues. *The Lancet* 379(9819): 943-952, 2012.

Batouli S, Miura M, Brahim J, Tsutsui TW, Fisher LW, Gronthos S, et al.: Comparison of stem-cell-mediated osteogenesis and dentinogenesis. *Journal of dental research* 82(12): 976-981, 2003.

Beertsen W, McCulloch CA, Sodek J: The periodontal ligament: a unique, multifunctional connective tissue. *Periodontology 2000* 13(1): 20-40, 1997.

Bianco P, Robey PG: Stem cells in tissue engineering. *Nature* 414(6859): 118-121, 2001.

Bohl KS, Shon J, Rutherford B, Mooney DJ: Role of synthetic extracellular matrix in development of engineered dental pulp. *Journal of Biomaterials Science, Polymer Edition* 9(7): 749-764, 1998.

Bolton K, Segal D, McMillan J, Sanigorski A, Collier G, Walder K: Identification of secreted proteins associated with obesity and type 2 diabetes in Psammomys obesus. *International journal of obesity* 33(10): 1153-1165, 2009.

Bružauskaitė I, Bironaitė D, Bagdonas E, Bernotienė E: Scaffolds and cells for tissue regeneration: different scaffold pore sizes—different cell effects. *Cytotechnology* 68(3): 355-369, 2016.

Cha Y, Jeon M, Lee HS, Kim S, Kim SO, Lee JH, et al.: Effects of In Vitro Osteogenic Induction on In Vivo Tissue Regeneration by Dental Pulp and Periodontal Ligament Stem Cells. *Journal of endodontics* 41(9): 1462-1468, 2015.

Chalisserry EP, Nam SY, Park SH, Anil S: Therapeutic potential of dental stem cells. *Journal of tissue engineering* 8: 1-17, 2017.

Chen G, Ushida T, Tateishi T: Scaffold design for tissue engineering. *Macromolecular Bioscience* 2(2): 67-77, 2002.

Chueh L-H, Ho Y-C, Kuo T-C, Lai W-H, Chen Y-HM, Chiang C-P: Regenerative endodontic treatment for necrotic immature permanent teeth. *Journal of endodontics* 35(2): 160-164, 2009.

Conde CM, Demarco FF, Casagrande L, Alcazar JC, Nör JE, Tarquinio SB: Influence of poly-L-lactic acid scaffold's pore size on the proliferation and differentiation of dental pulp stem cells. *Brazilian dental journal* 26(2): 93-98, 2015.

Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, et al.: Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. *Journal of endodontics* 34(8): 962-969, 2008.

Cvek M: Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. *Dental Traumatology* 8(2): 45-55, 1992.

Diogenes A, Henry MA, Teixeira FB, Hargreaves KM: An update on clinical regenerative endodontics. *Endodontic Topics* 28(1): 2-23, 2013.

Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM: Regenerative endodontics: a way forward. *The Journal of the American Dental Association* 147(5): 372-380, 2016.

Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K: Stem cells in dentistry–part I: stem cell sources. *Journal of prosthodontic research* 56(3): 151-165, 2012.

Eramo S, Natali A, Pinna R, Milia E: Dental pulp regeneration via cell homing. *International endodontic journal* 51(4): 405-419, 2018.

Galler KM, Hartgerink JD, Cavender AC, Schmalz G, D'Souza RN: A customized self-assembling peptide hydrogel for dental pulp tissue engineering. *Tissue Engineering Part A* 18(1-2): 176-184, 2012.

Gilbert TW, Sellaro TL, Badylak SF: Decellularization of tissues and organs. *Biomaterials* 27(19): 3675-3683, 2006.

Hu K, Olsen BR: The roles of vascular endothelial growth factor in bone repair and regeneration. *Bone* 91: 30-38, 2016.

Hu L, Liu Y, Wang S: Stem cell-based tooth and periodontal regeneration. *Oral diseases* 24(5): 696-705, 2018.

Hubbell JA: Biomaterials in tissue engineering. *Bio/technology* 13(6): 565-576, 1995.

Hutmacher DW: Scaffold design and fabrication technologies for engineering tissues—state of the art and future perspectives. *Journal of Biomaterials Science, Polymer Edition* 12(1): 107-124, 2001.

Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, et al.: Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. *Tissue Engineering Part A* 17(15-16): 1911-1920, 2011.

Iohara K, Murakami M, Takeuchi N, Osako Y, Ito M, Ishizaka R, et al.: A novel combinatorial therapy with pulp stem cells and granulocyte colony-stimulating factor for total pulp regeneration. *Stem cells translational medicine* 2(7): 521-533, 2013.

Isaka J, Ohazama A, Kobayashi M, Nagashima C, Takiguchi T, Kawasaki H, et al.: Participation of periodontal ligament cells with regeneration of alveolar bone.

Journal of periodontology 72(3): 314-323, 2001.

Karimbux NY, Nishimura I: Temporal and spatial expressions of type XII collagen in the remodeling periodontal ligament during experimental tooth movement. *Journal of dental research* 74(1): 313-318, 1995.

Keane TJ, Londono R, Turner NJ, Badylak SF: Consequences of ineffective decellularization of biologic scaffolds on the host response. *Biomaterials* 33(6): 1771-1781, 2012.

Kim JY, Xin X, Moioli EK, Chung J, Lee CH, Chen M, et al.: Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. *Tissue Engineering Part A* 16(10): 3023-3031, 2010.

Kling M, Cvek M, Mejare I: Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors. *Dental Traumatology* 2(3): 83-89, 1986.

Kuzu I, Bicknell R, Harris A, Jones M, Gatter K, Mason D: Heterogeneity of vascular endothelial cells with relevance to diagnosis of vascular tumours. *Journal of clinical pathology* 45(2): 143-148, 1992.

Lambrichts I, Driesen RB, Dillen Y, Gervois P, Ratajczak J, Vangansewinkel T, et al.: Dental pulp stem cells: their potential in reinnervation and angiogenesis by using scaffolds. *Journal of endodontics* 43(9): S12-S16, 2017.

Liu X, Chen W, Zhang C, Thein-Han W, Hu K, Reynolds MA, et al.: Co-seeding human endothelial cells with human-induced pluripotent stem cell-derived mesenchymal stem cells on calcium phosphate scaffold enhances osteogenesis and vascularization in rats. *Tissue Engineering Part A* 23(11-12): 546-555, 2017.

Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25(4): 402-408, 2001.

Lu H, Hoshiba T, Kawazoe N, Chen G: Comparison of decellularization techniques for preparation of extracellular matrix scaffolds derived from three-dimensional cell culture. *Journal of Biomedical Materials Research Part A* 100(9): 2507-2516, 2012.

Matsubara H, Hogan DE, Morgan EF, Mortlock DP, Einhorn TA, Gerstenfeld LC: Vascular tissues are a primary source of BMP2 expression during bone formation induced by distraction osteogenesis. *Bone* 51(1): 168-180, 2012.

McKee MD, Zalzal S, Nanci A: Extracellular matrix in tooth cementum and mantle dentin: localization of osteopontin and other noncollagenous proteins, plasma proteins, and glycoconjugates by electron microscopy. *The Anatomical record* 245(2): 293-312, 1996.

Nakashima M, Iohara K, Murakami M, Nakamura H, Sato Y, Ariji Y, et al.: Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. *Stem cell research & therapy* 8(1): 61, 2017.

Narayanan AS: Connective tissues of the periodontium: a summary of current work. *Collagen and related research* 3(1): 25-31, 1983.

Nene KS, Bendgude V: Prognosis of replanted avulsed permanent incisors: A systematic review. *International Journal of Pedodontic Rehabilitation* 3(2): 87-98, 2018.

Porzionato A, Stocco E, Barbon S, Grandi F, Macchi V, De Caro R: Tissue-engineered grafts from human decellularized extracellular matrices: a systematic review and future perspectives. *International journal of molecular sciences* 19(12): 4117, 2018.

Qu T, Jing J, Jiang Y, Taylor RJ, Feng JQ, Geiger B, et al.: Magnesium-containing nanostructured hybrid scaffolds for enhanced dentin regeneration. *Tissue Engineering Part A* 20(17-18): 2422-2433, 2014.

Romanos GE, Asnani KP, Hingorani D, Deshmukh VL: PERIOSTIN: role in formation and maintenance of dental tissues. *Journal of cellular physiology* 229(1): 1-5, 2014.

Schmalz G, Smith AJ: Pulp development, repair, and regeneration: challenges of the transition from traditional dentistry to biologically based therapies. *Journal of endodontics* 40(4): S2-S5, 2014.

Sharpe PT: Dental mesenchymal stem cells. *Development* 143(13): 2273-2280, 2016.

Son H, Jeon M, Choi HJ, Lee HS, Kim IH, Kang CM, et al.: Decellularized human periodontal ligament for periodontium regeneration. *PLoS One* 14(8): e0221236, 2019.

Song IS, Han YS, Lee J-H, Um S, Kim HY, Seo BM: Periodontal ligament stem cells for periodontal regeneration. *Current Oral Health Reports* 2(4): 236-244, 2015.

Song JS, Kim SO, Kim SH, Choi HJ, Son HK, Jung HS, et al.: In vitro and in vivo

characteristics of stem cells derived from the periodontal ligament of human deciduous and permanent teeth. *Tissue engineering. Part A* 18(19-20): 2040-2051, 2012.

Song JS, Takimoto K, Jeon M, Vadakekalam J, Ruparel NB, Diogenes A: Decellularized Human Dental Pulp as a Scaffold for Regenerative Endodontics. *Journal of dental research* 96(6): 640-646, 2017.

Srinivasan S, Jayasree R, Chennazhi K, Nair S, Jayakumar R: Biocompatible alginate/nano bioactive glass ceramic composite scaffolds for periodontal tissue regeneration. *Carbohydrate Polymers* 87(1): 274-283, 2012.

Stross W, Warnke R, Flavell D, Flavell S, Simmons D, Gatter K, et al.: Molecule detected in formalin fixed tissue by antibodies MT1, DF-T1, and L60 (Leu-22) corresponds to CD43 antigen. *Journal of clinical pathology* 42(9): 953-961, 1989.

Stucki U, Schmid J, Hämmerle CF, Lang NP: Temporal and local appearance of alkaline phosphatase activity in early stages of guided bone regeneration. A descriptive histochemical study in humans. *Clinical oral implants research* 12(2): 121-127, 2001.

Su WT, Wu PS, Ko CS, Huang TY: Osteogenic differentiation and mineralization of human exfoliated deciduous teeth stem cells on modified chitosan scaffold. *Materials science & engineering. C, Materials for biological applications* 41: 152-160, 2014.

Takeuchi N, Hayashi Y, Murakami M, Alvarez F, Horibe H, Iohara K, et al.: Similar in vitro effects and pulp regeneration in ectopic tooth transplantation by basic fibroblast growth factor and granulocyte-colony stimulating factor. *Oral diseases* 21(1): 113-122, 2015.

Thomson TS, Berry JE, Somerman MJ, Kirkwood KL: Cementoblasts maintain expression of osteocalcin in the presence of mineral trioxide aggregate. *Journal of endodontics* 29(6): 407-412, 2003.

Traweek ST, Kandalaft PL, Mehta P, Battifora H: The Human Hematopoietic Progenitor Cell Antigen (CD 3 4) in Vascular Neoplasia. *American journal of clinical pathology* 96(1): 25-31, 1991.

Van Hassel HJ: Physiology of the human dental pulp. *Oral Surgery, Oral Medicine, Oral Pathology* 32(1): 126-134, 1971.

Villarreal-Ramírez E, Moreno A, Mas-Oliva J, Chávez-Pacheco JL, Narayanan AS, Gil-Chavarría I, et al.: Characterization of recombinant human cementum protein 1 (hrCEMP1): primary role in biomineralization. *Biochem Biophys Res Commun* 384(1): 49-54, 2009.

Weissman IL, Anderson DJ, Gage F: Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. *Annual review of cell and developmental biology* 17(1): 387-403, 2001.

Yen A-H, Yelick P: Dental tissue regeneration–a mini-review. *Gerontology* 57(1): 85-94, 2011.

Yen AH, Sharpe PT: Regeneration of teeth using stem cell-based tissue engineering. *Expert opinion on biological therapy* 6(1): 9-16, 2006.

Zhu W, Liang M: Periodontal ligament stem cells: current status, concerns, and future prospects. *Stem cells international* 2015: 1-11, 2015.

국문요약

치아 조직 재생에 활용된 사람 치아 탈세포 지지체의 *in vivo* 평가

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지도교수 : 송 제 선

치주인대 및 치수의 재생은 손상된 치아의 회복에서 매우 중요하다. 기존의 치료 방법은 손상된 조직의 제거 및 대체로 진행되며, 이는 치아 생활력의 소실로 이어진다. 치아 생활력의 소실은 불량한 치아 예후로 이어질 수 있으며, 특히 미성숙 영구치의 경우, 치아의 발육이 중단된다. 치아의 생활력을 회복하고 생물학적 기능을 유지하기 위하여 치수조직과 치주인대조직의 재생과 관련된 연구가 활발히 진행되고 있으며, 조직 재생 측면에서 지지체는 중요한 요소 중 하나이다. 이에 본 연구는 영구치 탈세포 지지체를 이용한 치주인대 및 치수 조직 재생을 확인하려고 한다.

기존에 확인되었던 효과적인 탈세포 방법을 이용하여 영구치 탈세포 지지체를 제작하였다. 사람의 영구치에서 획득한 치수줄기세포와 치주인대줄기세

포를 탈세포 지지체에 접종하여 배양한 후, 면역억제된 쥐에 이식하여 9주간 조직재생을 유도하였다. 이후 이식체를 분리하여 재생된 조직에 대한 조직학적 분석, 면역화학염색법, 실시간 중합효소 연쇄반응분석을 시행하였다.

치주인대줄기세포의 경우, 접종한 세포들이 탈세포 지지체의 치주인대 세포외 기질 내부까지 투과하여 생존, 분화한 것을 관찰 할 수 있었고, CP23, Col I, Col XII, OC, ALP의 높은 발현이 관찰되어, 백악질/치주인대 복합체의 재생 가능성을 기대할 수 있었다.

치수줄기세포 역시 접종한 세포들은 탈세포 지지체의 치수조직 세포외 기질에서 생존, 분화하는 것을 확인 할 수 있었고, DSPP, DMP1, NES, Col I, ALP, CD31, CD34의 높은 발현이 관찰되었다. 또한 재혈관화와 관련된 유전자의 발현과 신생 경조직의 생성은 치수 조직 재생의 가능성을 보여주었다.

본 연구를 통해 치주인대줄기세포 및 치수줄기세포를 이용한 치주인대 및 치수조직 재생에 영구치 탈세포 지지체의 활용 가능성을 확인할 수 있었고, 이를 치아조직의 재생에 대한 새로운 치료방법 및 연구에 응용할 수 있을 것으로 기대한다.

핵심되는 말: 탈세포 지지체, 치수줄기세포, 치주인대줄기세포, 조직재생