The effect of soft tissue substitutes in gingival thickening: histologic and volumetric analyses in dogs

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The effect of soft tissue substitutes in gingival thickening: histologic and volumetric analyses in dogs

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The Doctoral Dissertation
submitted to the Department of Dentistry
and the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Ph.D. in Dental Science

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June 2019

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June 2019

감사의 글

가장 먼저, 본 연구가 학위 논문으로 완성이 되기까지 여러 면에서 부족한 저를 사랑과 격려의 마음으로 지도해주시고 이끌어주신 정의원교수님께 진심으로 감사드립니다. 아울러, 저의 치주과 수련 및 대학원 재학 중의 은사님들이신 채중규, 조규성, 최성호, 김창성, 이중석, 차재국, 백정원교수님과 바쁘신 와중에도 저의 학위 논문 심사를 맡아주신 김성태, 김영택교수님, 그리고 치주를 전공함에 항상 많은 영감을 주시는 김종관교수님께도 깊이 감사를 드립니다.

실험을 진행하는 데에 많은 도움을 준 연구원 선생님들, 그리고 함께 공부한 의국원들에게도 감사의 마음을 전합니다.

아낌없는 사랑으로 제게 항상 힘을 주는 사랑하는 아내 윤희, 그리고 사랑하는 두 딸 윤서와 민서에게 감사의 마음과 사랑의 뜻을 전합니다. 그리고 항상 넘치는 사랑과 격려의 마음으로 응원을 해주신 양가 부모님과 양가 형제, 자매들에게도 사랑과 감사의 마음을 전합니다.

2019년 6월

저자 송영우

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Abstract

The effect of soft tissue substitutes in gingival thickening: histologic and volumetric analyses in dogs

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Purpose: To evaluate the histologic and volumetric changes of gingival tissues following grafting with collagen-based matrices at labial aspect of teeth in canines.

Methods: Gingival augmentation was performed in the mandibular incisor area using two types of xenogeneic cross-linked collagen matrices (CCMs), bovine CCM for BCCM group and porcine CCM for PCCM group, whereas the contralateral sides remained untreated (B-control group and P-control group). Descriptive histology, histometric and volumetric analyses were performed after 12 weeks. For statistical comparison between each test group and respective control group, paired t-test was used for histometric analysis, and repeated-measured analysis of variance was used for volumetric analysis ($p<0.05$).

Results: An increased number of rete pegs and an enhanced formation of new blood vessels were observed at both grafted sites compared to the corresponding control sites. There was statistically significant gain of horizontal thickness only in BCCM group (1.36 ± 0.27mm vs. 1.26 ± 0.34mm; $p<0.05$) compared to the B-control groups.

Conclusion: BCCM was effective for gingival augmentation in terms of horizontal thickness at the labial aspect of teeth at 12 weeks post-surgery.

Keywords: cross-linked collagen matrix; soft tissue augmentation; descriptive histology; histometric analysis; volumetric analysis

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I. INTRODUCTION

Soft tissue grafting procedures have been usually performed to increase the width of keratinized tissue, the thickness of gingival tissues as well as for recession coverage. Controversy exists in terms of the necessity of a certain amount of keratinized tissue around teeth for maintaining periodontal health and the soft tissue lining. Previous studies reported a sufficient amount of keratinized tissue to be a critical element (Kim and Neiva, 2015; Kothiwale et al., 2016; Lang and Loe, 1972). Other studies, however, demonstrated that periodontal health was not significantly correlated with the thickness/width of keratinized tissue (Dorfman et al., 1982; Kennedy et al., 1985; Wennstrom and Lindhe, 1983a, 1983b). In a recent consensus report, it was concluded that the gingival thickness

and width are not necessary for maintaining a healthy periodontium, when oral hygiene care is optimized (Jepsen et al., 2018). Since it is not always possible to obtain a plaque-free environment in a clinical situation, a sufficient amount of keratinized tissue might be necessary to overcome the patient's deficiency of obtaining a favorable oral hygiene.

It is also known that a thick gingival biotype is critical to prevent gingival recession not associated with plaque, such as orthodontic movement of teeth (Rasperini et al., 2015; Wennstrom et al., 1987; Zucchelli and Mounssif, 2015). In such a case, recessions could be prevented by two means (Hwang and Wang, 2006): firstly, a thicker soft tissue contains a higher volume of extracellular matrix and collagen networks allowing to withstand contraction and collapse; secondly, an increased gingival vascularity creates enhanced oxygen supply leading to an enhanced clearance of toxic products and growth factor migration. Consequently, a thicker gingiva has a higher healing potential and resistance to recession.

Furthermore, the gingival biotype is one of the critical prognostic factors for root coverage procedures (Rasperini et al., 2015). It has been reported that the extent of root coverage is improved as the gingival thickness increases (Hwang and Wang, 2006). Traditional autogenous grafts demonstrated clinically predictable outcomes at tooth sites (Cairo et al., 2014; Tatakis et al., 2015). However, involving an intraoral donor site may cause patients' discomfort due to multiple operating sites and relatively long operating times (Fickl et al., 2014; Zucchelli et al., 2010).

In order to overcome disadvantages of autogenous tissue, various collagen matrices were evaluated in pre-clinical and clinical studies demonstrating a gain in soft tissue thickness/width and a reduced patient morbidity and operating time (Jepsen et al., 2013; Rocchietta et al., 2012; Schmitt et al., 2016; Thoma et al., 2012a; Vignoletti et al., 2011). However, previous data revealed that augmented soft tissue with collagen matrices showed a shrinkage of more than 50% over 3.5 years of observation (Rothamel et al., 2005; Schwarz et al., 2006; Simion et al., 2012). In order to overcome the relatively high

shrinkage rate, cross-linked collagen matrices (CCMs) were proposed. Various pre-clinical and clinical studies demonstrated the efficacy of xenogeneic CCMs (Cha et al., 2017; Thoma et al., 2011; Thoma et al., 2010; Thoma et al., 2012b; Zahedi et al., 1998). This was based on a reported slower degradation and an enhanced volume stability. Still, the number of studies using collagen matrices at tooth sites is limited.

The aim of this pre-clinical study was to evaluate histological and volumetric changes following grafting with two collagen-based matrices with different types of cross-linking at the labial aspect of teeth in canines.

II. MATERIALS AND METHODS

The manuscript was written following the Animal Research: Reporting In Vivo Experiments (ARRIVE) Guidelines Checklist (Kilkenny et al., 2010).

1. Ethical statement

For the experiment, animal selection, management and experimental protocol were approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Republic of Korea (Permission no. 2013-0317-2).

2. Experimental animals

Six healthy beagle dogs with the mean age of 12 to 15 months old and the mean body weight of 10 ~ 15 kg were used. The animals were raised individually under standard laboratory conditions and proper feeding, and they had sound periodontium with permanent dentition. Sample size was calculated by power calculation with the significance level (α) of 5% and power ($1-\beta$) of 90%, according to the results from previous studies (Thoma et al., 2011; Thoma et al., 2010). The animals were housed under constant room temperature (15-20℃) and humidity (>30%).

3. Experimental materials

Two different types of xenograft CCMs were used:

• BCCM: bovine-derived CCM (Collagen Graft®; Genoss, Suwon, Republic of Korea), a double-layered matrix with chemically cross-linked type I collagen derived from bovine tendon

• PCCM: porcine-derived CCM (a prototype of double-layered collagen-based material; SK Bioland, Cheongju, Republic of Korea), composed of type I collagen derived from porcine pericardium with dehydrothermal cross-linking.

Both materials were double-layered with a porous structure on one side and a compact structure on the other side.

4. Surgical intervention

For the surgery, the dogs were anesthetized with intramuscular injection of medetomidine (0.75mg/kg; Tomidin®, Provet veterinary products Ltd, Istanbul, Turkey) and intravenous injection of alfaxalone (2mg/kg; Jurox, Rutherford NSW, Australia). In addition, inhalation anesthesia with isofluran (Forane®, Choongwae Pharmaceutical, Seoul, Korea) and local infiltration anesthesia was performed with lidocaine (2% lidocaine HCL with epinephrine 1:80,000; Kwangmyung Pharm., Seoul, Korea) on surgical site. Scaling and plaque control were conducted before surgery. Crevicular incision was performed on the first incisor area of PCCM group and the third incisor area of BCCM group. Vertical incision was made on attached gingiva underneath marginal gingiva at mesiolabial side of the first incisor area and distolabial side of the third incisor area, forming a subperiosteal pouch to insert materials (Fig. 1a and 1b). Orban interdental knife was inserted carefully into the incision line and proceeded to gingival sulcus to form a gingival tunnel, allowing to advance the material coronally. Subsequently, the following four treatment modalities were applied in a randomized split-mouth design;

• BCCM group: bovine-derived CCM, applied on the labial gingiva of the third incisor area.
• PCCM group: porcine-derived CCM, applied on the labial gingiva of the first incisor area.
• B-control group: negative control site at contralateral side of BCCM group.
• P-control group: negative control site at contralateral side of PCCM group.

For that purpose, the materials were trimmed to 6mm x 4mm x 2mm (length x width x depth) (BCCM) (Fig. 1c) and to 4mm x 4mm x 2mm (length x width x depth) (PCCM) (Fig. 1d). On the contralateral side of BCCM group and PCCM group, no surgical interventions were performed (B-control group and P-control group respectively). Primary closure was achieved with resorbable silk material (Monosyn® 6-0 Glyconate Monofilament, B. Braun Tuttlingen, Germany). The dogs received antibiotic of cefazolin sodium (20mg/kg; Yuhan, Seoul, Korea) and non-steroidal anti-inflammatory drugs of meloxicam (0.2mg/kg; Mobic®, Boehringer Ingelheim, Ingelheim, Germany) administration for 7 days after the surgery. Surgical sites were cleaned daily by irrigation with 0.2% chlorhexidine (Hexamedine; Bukwang Pharmaceutical, Seoul, Korea). Suture materials were removed 10 days after the surgery. The animals were sacrificed 12 weeks after the operation by an overdose of 3% sodium pentobarbital, and lower jaws were dissected to obtain block specimens with intact soft tissues.

5. Descriptive histology and histometric analyses

The resected specimens were trimmed and embedded in paraffin after decalcification, and the center-most section was chosen for analyses. The slides were dyed with hematoxylin and eosin staining and Masson's trichrome staining. Light microscope (BX51; Olympus Research Systems, Tokyo, Japan), equipped with a camera, was used for histologic observation. A histometric assessment was performed with a computer

software (Photoshop® CS6, Adobe System, San José, CA, USA) by a single investigator (Y.W.S.), blinded to the surgical procedure and group allocations. The same investigator measured twice in 2-week-interval. The following parameters were measured as primary variables (Fig. 2a);

• Horizontal thickness (T, mm): mean of the data measured at five different levels (1.0mm and 0.5mm above the cementoenamel junction (CEJ), CEJ level, and 0.5mm and 1.0mm below the CEJ)

- Tts: mean thickness of total soft tissue (from the sulcular or junctional epithelium to the keratinized epithelium)

- Tct: mean thickness of total connective tissue

- Tdct: mean thickness of dense connective tissue

• Number of rete pegs within the region of interest (ROI; 3mm in height x 1mm in width)

- Rete pegs underneath the keratinized epithelium

- Rete pegs underneath the sulcular epithelium and junctional epithelium

6. Dental impressions

Dental impressions at all five time points (pre-surgery, 10 days post-surgery (suture-removal), 4 weeks post-surgery, 8 weeks post-surgery and 12 weeks post-surgery) were obtained from the mandibles using polyvinylsiloxane impression materials (Aquasil Ultra LV® and Aquasil Ultra XLV®, Dentsply DeTrey, Konstanz, Germany). Individualized trays, pre-fabricated for study-purposes and made of a self-cured acrylic resin (Formatray® Kerr Manufacturing, Romulus, MI, USA), were used for impression taking. Master casts were poured out of dental stone (GC Fujirock® type 4, GC Corporation, Tokyo, Japan) and digitized using a dental scanner (Imetric 3D SA, Courgenay, Switzerland).

7. Volumetric analysis

The resulting STL-files were subsequently analyzed using a software (SMOP, Swissmeda, Zurich, Switzerland) allowing for superimposition with respect to static points (Fig. 2b). The calculation of volumetric changes at the sites was performed by an experienced examiner (T.W.) unaware of the treatment groups at the University of Zurich under GLP like conditions (Fig. 2c). The region of interest (ROI) was manually selected as follows: the apico-coronal dimension was determined 0.5mm apically from the gingival margin and extended 1.5mm in an apical direction (Fig. 2d). The mesio-distal dimension was defined with a clearance of 0.5mm to the mesial and distal adjacent teeth. The mean distance (MD, mm) within the selected area between the selected STL surface and the pre-surgical baseline STL was computed by the software, and following parameters were measured:

- *Δ10D: MD between the time points of 10 days post-surgery and pre-surgery*
- *Δ4W: MD between the time points of 4 weeks post-surgery and pre-surgery*
- *Δ8W: MD between the time points of 8 weeks post-surgery and pre-surgery*
- *Δ12W: MD between the time points of 12 weeks post-surgery and pre-surgery*

For direct comparison of the different sites and treatment modalities, MD was defined as the measured volume difference per measured area of ROI (MD[mm] = vol [mm^3]/area [mm^2]).

8. Experimental outcomes

The primary outcome variable was the change in histometric parameters after gingival augmentation. Descriptive histology and volume change over time were assessed as secondary outcomes.

9. Statistical analysis

Statistical analysis was performed using a computer software (SPSS version 23, IBM, Armonk, NY, USA). Histometric measurements between BCCM group and B-control group and between PCCM and P-control group were assessed using a paired t-test, and repeated-measured analysis of variance was used for analyzing mean volume differences compared to pre-surgical state over time. The unit for statistical analysis was the dog, and the level of significance was set $p < 0.05$.

III. RESULTS

1. Clinical outcomes

Suture-removal of two dogs was postponed for a week due to a delayed healing. The further healing thereafter was uneventful. Other than that, all experimental animals stayed healthy until the sacrifice, without any specific systemic or local complications.

2. Descriptive histology

Both BCCM group and PCCM group showed prominent changes in morphology of rete pegs compared to control groups. Rete pegs were of narrower and deeper shape in both BCCM and PCCM groups compared to the respective control groups. Moreover, rete pegs were mainly observed underneath the keratinized epithelium in control groups. In the two test groups, an increased number of rete pegs were also present underneath the sulcular and junctional epithelium.

In a few samples in group BCCM, remnants of the matrix were encapsulated by newly formed collagen fibers and embedded within dense connective tissue with an increased vascularization. None of the PCCM samples showed remnants of the matrix network. (Fig. 3).

3. Histometric analyses

The results of the histometric analysis are represented in Table 1. For mean Tts, Tct and Tdct values, both BCCM and PCCM groups demonstrated an increase in thickness

compared to the respective control groups at 12 weeks (BCCM: + 0.10mm, + 0.13mm and + 0.15mm; PCCM: + 0.03mm, + 0.05mm and + 0.07mm). Statistical significance was only observed for group BCCM compared to B-control group ($p<0.05$). In both test groups, the number of rete pegs increased underneath the keratinized epithelium (BCCM: $p<0.05$; PCCM: $p<0.05$) and underneath the sulcular epithelium and junctional epithelium (BCCM: $p<0.05$; PCCM: $p>0.05$) compared to the control groups (Fig. 4).

4. Volumetric analysis

Means, standard deviations and statistical significances for the volumetric analysis are displayed in Table 2. Both test groups showed an increased thickness between pre-surgery and all follow-up time-points compared to the control groups. Mean increases up to 12 weeks post-surgery (Δ12W*)* were 0.14 ± 0.11mm in BCCM group and 0.27 ± 0.13mm in PCCM group. No statistical differences were calculated between groups at any time-point ($p>0.05$).

IV. DISCUSSION

This in vivo preclinical study aimed to assess histological and volumetric changes in soft tissue thickness, 12 weeks after gingival grafting at the labial aspect of teeth. The BCCM showed a significantly greater gingival thickness (based on histometric analysis) compared to the control group. Total soft tissue, total connective tissue and dense connective tissue thickness were enhanced by 8.03%, 11.96%, 18.14% in the BCCM group and by 2.96%, 6.11%, 11.26% in the PCCM group, always in comparison to the respective control groups. Based on the volumetric analysis, both, the BCCM and PCCM group, demonstrated a decrease in thickness over time (12 weeks post-surgery, compared to suture-removal), with a shrinkage rate of 52.33% in the BCCM group and 30.57% in the PCCM group . These results are consistent with the ones reported in previous studies (Rothamel et al., 2005; Schmitt et al., 2016; Thoma et al., 2011; Thoma et al., 2010), and imply a change in the gingival biotype following grafting with CCMs.

A slower biodegradation of CCM contributes to the maintenance of augmented volume for a longer time. In contrast, it may inhibit tissue integration based on an increased inflammatory reaction, causing a delayed wound healing and more postoperative discomfort (Bornstein et al., 2007; Schwarz et al., 2008). Since the histological observation was based 12 weeks after the procedure, a late time-point after soft tissue grafting, both types of CCM used in this study demonstrated favorable biological integration without apparent clinical complications, including no inflammatory reaction. In the majority of the BCCM group slides, remnants of the CCM were present, whereas no remnants of the matrix in the PCCM group were found. This result is consistent with previous studies showing that CCM may not be fully resorbed after 12 weeks (Rothamel et al., 2005; Thoma et al., 2011). This conflicting result between BCCM group and PCCM group can be explained by a modified technique for cross-linking.

Cross-linking of PCCM was achieved by dehydrothermal method, and BCCM was chemically cross-linked. Dehydrothermal cross-linking has shown better performance with less foreign body reaction than chemical cross-linking, which may induce a cytotoxic response (Haugh et al., 2009; Rothamel et al., 2014; Rothamel et al., 2005). Using this concept, a faster tissue integration of PCCM might have induced a faster biodegradation compared to the BCCM group, resulting in no matrix remnants present after 12 weeks. However, in contrast to previous reports, BCCM did not show an increased rate of complications during healing, neither clinically nor histologically, despite a slower biodegradation.

The BCCM group showed significantly enhanced Tts and Tct values compared to the respective control group based on the histometric analysis. Since the CCM was applied above the mucogingival junction, where dense connective tissue is mainly located, Tdct increased significantly. Considering that the main purpose of soft tissue augmentation is enhancing the gingival thickness, BCCM was successful in increasing the soft tissue thickness, to a similar extent as the results from a previous study (Thoma et al., 2011). Interestingly, the results of BCCM demonstrated that the enhanced gingival thickness was maintained after a soft tissue remodeling process, involving a maturation with formation of collagen fibers (Ramfjord et al., 1966; Selvig et al., 1988). In the PCCM group, the gingival thickness increased without statistical significance compared to the respective control group.

The number of rete pegs increased significantly in the BCCM group and the PCCM group compared to each control group. Previous studies have reported that narrower and deeper rete peg formation and an increased number might represent a mature healing condition and evidence that the matrix served as a scaffold to accelerate the healing process (Vignoletti et al., 2015). Rete pegs, also called epithelial ridges, separate the connective tissue portions from the epithelium. In general, rete pegs are observed mainly underneath the keratinized epithelium, however, all of the histologic slides in the BCCM

group and the PCCM group showed an increased number of rete pegs underneath the non-keratinized sulcular epithelium and the junctional epithelium. This finding is consistent with the results from previous studies (Cha et al., 2017; Vignoletti et al., 2015). Changes in the number and morphology of rete pegs represent a stronger resistance to forces applied on the gingiva (Grossman and Forbes, 1990). Morphological changes into a narrower and deeper shape and an increase in the number of rete pegs in both, the BCCM group and the PCCM group, led to an increase in surface area and a stronger anchorage between the epithelium and the connective tissue, thereby enhancing the mechanical properties to prevent gingival recession.

For the volumetric analysis, the casts were superimposed with an improved optical scanning method, as described earlier (Thoma et al., 2010). Both, BCCM and PCCM, enhanced the soft tissue volume compared to the respective control groups, but also demonstrated a decreasing volume over time. This loss of volume was probably due to a gradual relief of the post-operative swelling and biodegradation of the materials involving tissue integration (Schmitt et al., 2016; Thoma et al., 2010; Thoma et al., 2012b). The BCCM group showed a relatively consistent volume change over time, whereas in the PCCM group, a larger variation of the volume changes was evident. Calculating the shrinkage in a linear dimension, the rates in this study were similar to the ones from a previous study (Rothamel et al., 2005).

There were some limitations of this study. Firstly, to confirm the efficacy of the CCMs as an alternative to autogenous tissue, a positive control group (subepithelial connective tissue graft) could have been allocated. Due to limitations in terms of the number of available sites, no such control group was applied. Secondly, in order to allow for 3D measurements, impressions were taken and subsequently study casts scanned. One might consider using digital impression in the future to reduce possible inaccuracies based on these two steps (conventional impression, study cast).

V. CONCLUSION

Within the limitation of the present study, BCCM might have the potential to serve as an alternative for soft tissue augmentation at tooth sites. Further studies are needed to confirm the obtained data (enhanced volumetric stability of CCMs) and include additional clinical outcomes measures such as the extent of biological complications and patient morbidity as well as a comparison with autogenous grafts.

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TABLES

Table 1. Descriptive statistics of mean horizontal thickness measurements (mean ± standard deviation; mm).

	BCCM	B-control	PCCM	P-control
Tts	**1.36 ± 0.27***	1.26 ± 0.34	1.07 ± 0.30	1.04 ± 0.30
Tct	**1.19 ± 0.27***	1.06 ± 0.36	0.90 ± 0.30	0.85 ± 0.29
Tdct	**1.00 ± 0.30***	0.85 ± 0.33	0.72 ± 0.29	0.65 ± 0.24

* (bold): Significantly different from control group (p<0.05)

Table 2. Descriptive statistics of volume change at suture-removal, 4 weeks, 8 weeks and 12 weeks compared to pre-surgical state, measured as MD (mean ± standard deviation; mm).

	Δ10D	Δ4W	Δ8W	Δ12W
BCCM group	0.29 ± 0.24	0.22 ± 0.20	0.14 ± 0.21	0.14 ± 0.11
B-control group	0.25 ± 0.31	0.14 ± 0.17	0.05 ± 0.10	0.00 ± 0.00
PCCM group	0.39 ± 0.22	0.36 ± 0.15	0.33 ± 0.14	0.27 ± 0.13
P-control group	0.33 ± 0.27	0.24 ± 0.17	0.17 ± 0.08	0.19 ± 0.20

FIGURES

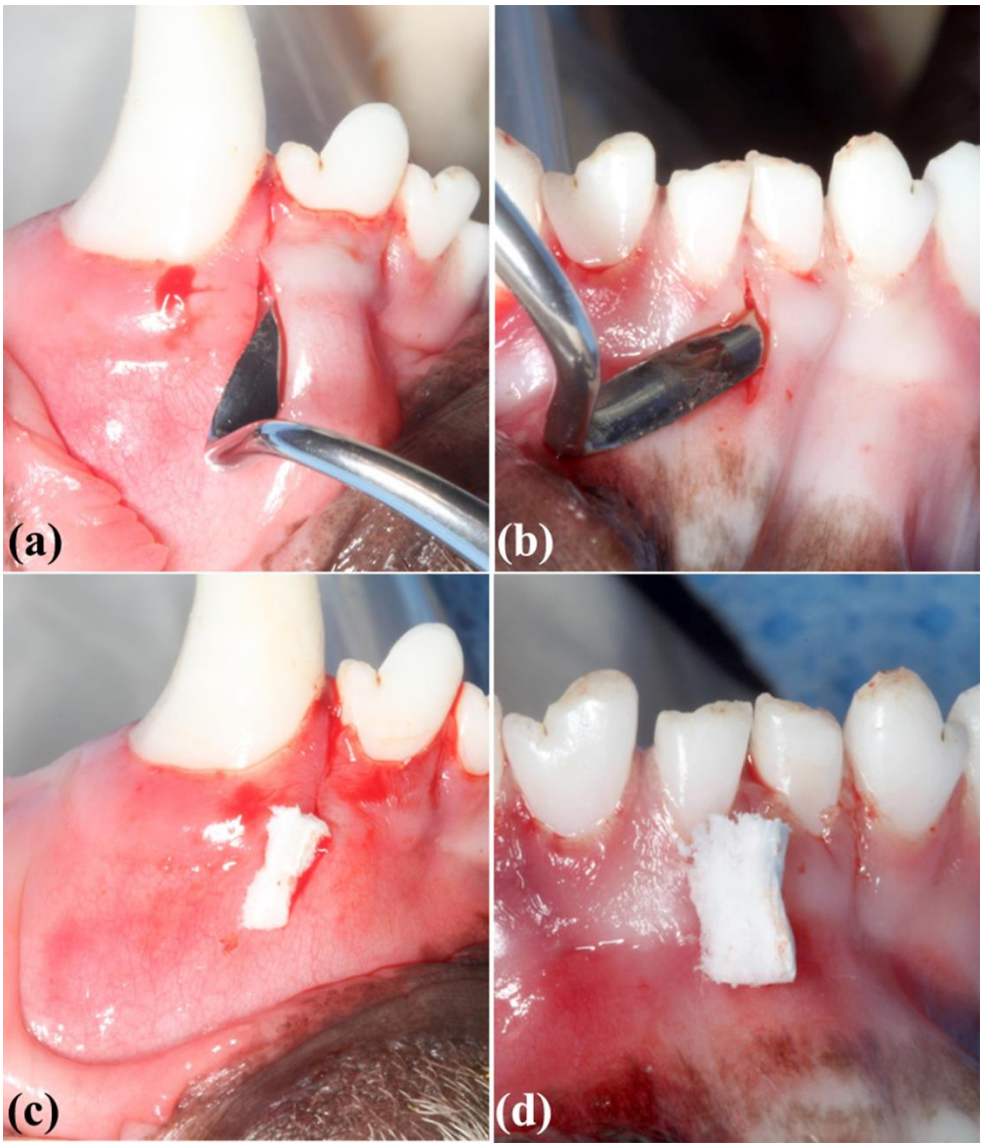

Figure 1. Clinical photographs of animal surgery.

Pouch formation in BCCM group (a) and PCCM group (b). Matrix insertion in BCCM group (c) and PCCM group (d).

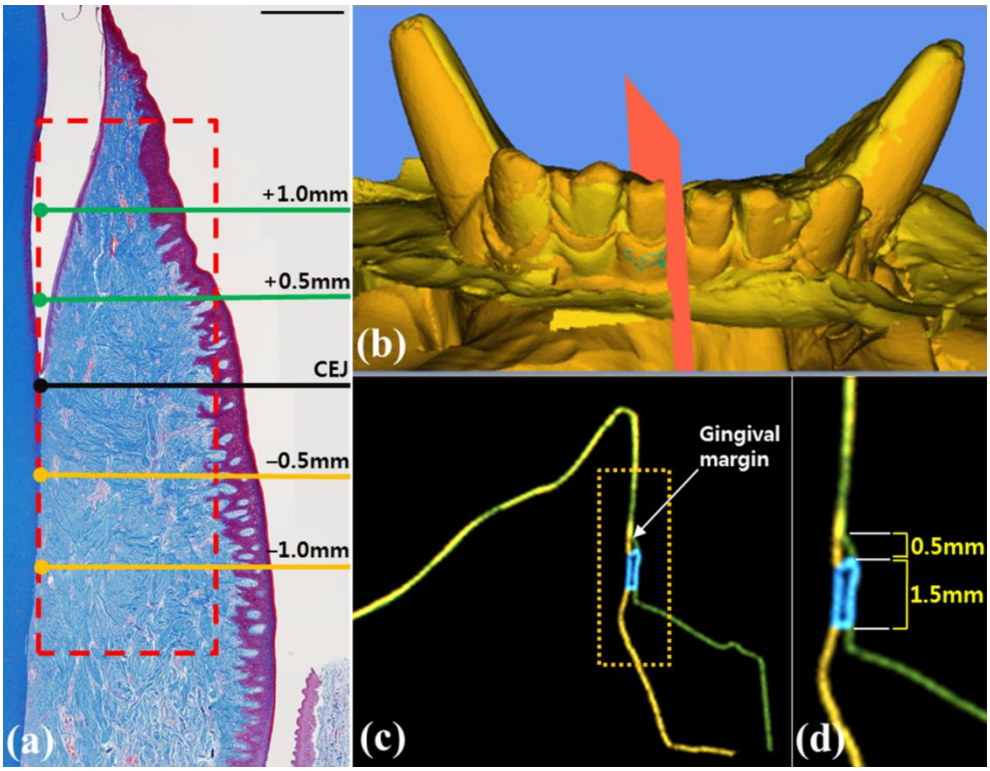

Figure 2. Histometric and volumetric measurements.

(a) Schematic drawing of histometric analysis. Black bar on the upper right border indicates scale bar of 500μm in length. Colored bars with single dot indicate levels CEJ (black), levels above CEJ (green) and levels below CEJ (yellow). Red-dotted rectangle indicates region of interest with 3mm in height and 1mm in width for counting the number of rete pegs. (b) Superimposition of optically scanned images. (c) Showing the sliced section for measuring the gap between the models for volume analysis. White arrow indicates gingival margin. (d) Magnified image of region of interest surrounded by orange-dotted rectangle in (c). Region of interest was set from 0.5mm apical to gingival margin to 2.0mm apical to gingival margin, which is marked in fluorescent blue, for measuring MD.

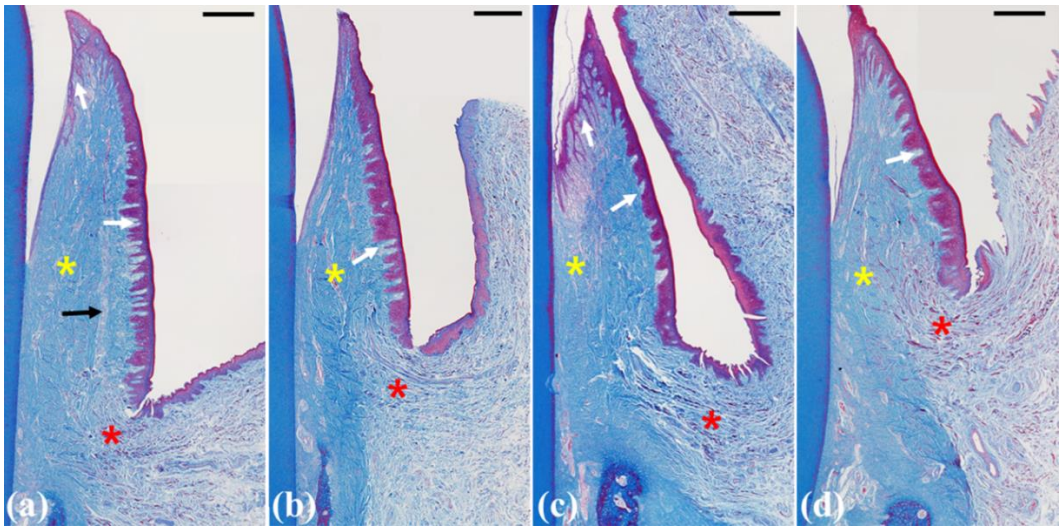

Figure 3. Descriptive histology.

(a) BCCM group. (b) B-control group. (c) PCCM group. (d) P-control group. Black bar on the upper right border indicates scale bar of 500μm in length. Colored asterisks indicate dense connective tissue (yellow) and loose connective tissue (red). Colored arrows indicate rete pegs (white) and cross-linked collagen matrix remnant (black).

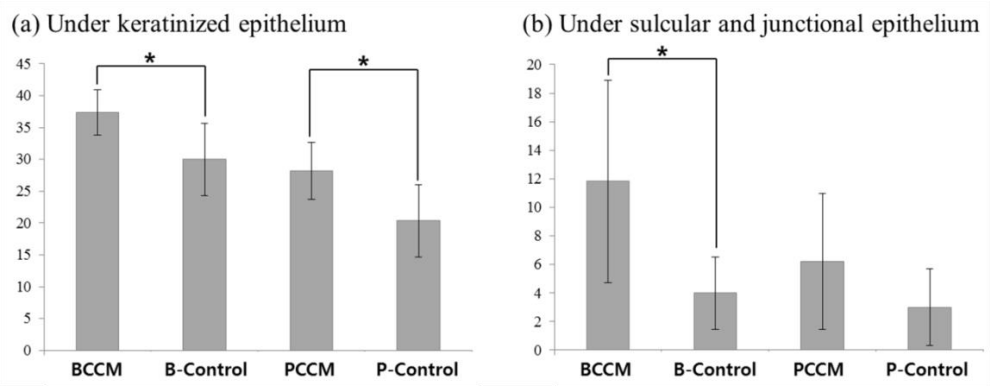

Figure 4. Number of rete pegs within the region of interest.

* (bold): statistical significance (p<0.05)

국문요약

연조직 대체재를 이용한 치은 이식술 시 치은 두께 증강 효과: 성견에서의 조직학적 및 부피계측학적 연구

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치은 이식술은 치아 주위 각화 조직의 두께와 폭을 증가시키고 퇴축된 치은 높이를 회복하는 데에 주로 이용되는 술식으로, 환자의 구강 내 공여부에서 채득한 자가 조직을 수여부에 이식하는 방식이 가장 널리 이용되고 있다. 자가 연조직 이식술은 높은 예지성과 장기적 안정성을 보인다는 장점이 있으나, 구강 내 두 군데 이상의 부위에서 수술이 진행되어야 하고, 술식의 난이도가 높다는 점이 환자와 술자 모두에게 단점으로 간주된다. 이러한 단점을 극복하고자 자가 연조직을 대신할 수 있는 동종 또는 이종 유래 재료들이 소개되어 왔고, 그 중 이종 유래 콜라겐 대체재는 유수의 기존 연구들에서 양호한 치은이식술 결과를 나타냈으나, 흡수 속도가 다소 빠름으로 인해 장기적 부피 안정성이 부족하다는 평가를 받고 있다. 재료의 흡수를 지연시키기 위해 콜라겐 섬유를 교차 결합 시키는 개념이 도입되어, 몇몇의 연구를 통해 결과가 보고되었으나, 치아 주위 연조직 두께 증강 면에서의 효과를 확인한 연구는 그 수가 아직 부족하다. 따라서 본 연구는, 성견의 하악 전치부 순측 치은에 교차 결합된 이종 유래

콜라겐 대체재를 이식한 후의 변화를 조직학적 그리고 부피계측학적으로 확인하는 데에 목적을 두었다.

두 종류의 서로 다른 이종 콜라겐 연조직 대체재를 시험군 이식 재료로 이용하였다. 성견 비글 6 마리를 대상으로 split-mouth 디자인을 통해 편측 하악 전치부에는 시험군 (BCCM 군과 PCCM 군)을, 반대측 하악 전치부에는 대조군 (B-control 군과 P-control 군)을 배정하였다. BCCM 군의 경우, 편측의 하악 제 3 절치에 가교 결합이 된 소 유래 콜라겐 대체재 (bovine-derived cross-linked collagen matrix, BCCM)를 6mm (높이) x 4mm (폭) x 2mm (두께)의 크기로 이식하였고, PCCM 군의 경우, 편측의 하악 제 1 절치에 가교 결합이 된 돼지 유래 콜라겐 대체재 (porcine-derived cross-linked collagen matrix, PCCM)를 4mm (높이) x 4mm (폭) x 2mm (두께)의 크기로 이식한 뒤, 각각 반대편 치아 부위의 대조군 (B-control 군과 P-control 군)과 비교하였다. 이식 수술 12 주 뒤 실험동물을 희생한 후, 조직학적 그리고 부피계측학적 평가를 시행하였다. 시편 염색 후 광학 현미경을 통해 조직학적 관찰을 진행하고, 전체 연조직 (상피층 + 결합조직층), 전체 결합조직층, 치밀 결합조직층의 두께를 측정하였으며, 각화 상피 하방과 열구 상피 및 접합 상피 하방의 상피 돌기의 수 역시 계측하였다. 술전과 술후 10 일, 4 주, 8 주, 12 주에 인상 채득하여 만든 석고 모형을 3D 스캔한 뒤, 이를 통해 얻은 영상들을 중첩하여 부피 계측도 시행하였다. 각각의 시험군과 대조군의 통계적 비교를 위해, 조직학적 계측치는 대응 표본 t 검정을, 부피 계측치는 반복 측정 분산 분석을 시행하였다 (p<0.05).

조직학적 관찰 결과, 각각의 대조군과 비교하였을 때, BCCM 군과 PCCM 군 모두 혈관 형성이 증가하였고, BCCM 군에서는 잔존 콜라겐 대체재가 관찰된 반면, PCCM 군에서는 잔존 대체재가 관찰되지 않았다. BCCM 군과 PCCM 군 모두 상피 돌기의 형태가 좁고 가느다란 형상으로 변화된 양상이

관찰되었고 그 수 역시 증가하였는데, 상피 돌기의 수는 각화 상피 하방 뿐만 아니라 열구 상피 및 접합 상피 하방에서도 증가한 양상이 확인되었다. 조직학적 계측 결과, 치은의 두께는 BCCM 군과 PCCM 군 모두 각각의 대조군과 비교하였을 때 증가하였으나, 통계적 유의 차는 BCCM 군에서만 확인되었다 (전체 연조직 두께: 1.36 ± 0.27 mm vs. 1.26 ± 0.34 mm; $p<0.05$). 이식 후 부피 변화 측면에서는 BCCM 군과 PCCM 군 모두 시간이 지남에 따라 부피가 감소하였고, BCCM 군과 PCCM 군 모두 각각의 대조군과 비교하여 이식 후 증가된 부피를 보였으나, 통계적으로 유의 차는 확인되지 않았다.

본 실험 결과는 가교 결합이 된 소 유래 콜라겐 대체재가 순면 치은 두께 증강 측면에서 효과적인 것으로 확인되었고, 치은 이식술 시 자가 연조직을 대체할 수 있는 가능성을 일부 확인하였다. 후속 연구를 진행함으로써, 본 실험 결과를 임상에 활용할 수 있도록 해야 할 것이다.

핵심되는 말: 가교 결합이 된 콜라겐 대체재, 연조직 이식술, 조직학적 관찰, 조직계측학적 평가, 부피계측학적 분석