Received: 27 December 2017 | Revised: 29 August 2018 | Accepted: 23 October 2018

DOI: 10.1111/jcpe.13034

WILEY Journal of Clinical Periodontology

ANIMAL EXPERIMENT

Soft tissue substitutes to increase gingival thickness: Histologic and volumetric analyses in dogs

Young Woo Song[1†] | Sungtae Kim[2†] | Tobias Waller[3] | Jae-Kook Cha[1] | Sung-Won Cho[4] | Ui-Won Jung[1] | Daniel S. Thoma[3]

[1]Department of Periodontology, Research Institute for Periodontal Regeneration, Yonsei University College of Dentistry, Seoul, Korea

[2]Department of Periodontology, School of Dentistry, Dental Research Institute, Seoul National University, Seoul, Korea

[3]Clinic of Fixed and Removable Prosthodontics and Dental Material Science, Center of Dental Medicine, University of Zurich, Zurich, Switzerland

[4]Division of Anatomy and Developmental Biology, Department of Oral Biology, Yonsei University College of Dentistry, Seoul, Korea

Correspondence
Ui-Won Jung, Department of Periodontology, Yonsei University College of Dentistry, Seoul, Korea.
Email: drjew@yuhs.ac
and
Sung-Won Cho, Division of Anatomy and Developmental Biology, Department of Oral Biology, Yonsei University College of Dentistry, Seoul, Korea.
Email: chosome1@yuhs.ac

Funding information
This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI15C1535).

Abstract

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

Materials and 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 ± 0.27 mm vs. 1.26 ± 0.34 mm; $p < 0.05$) 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, descriptive histology, histometric analysis, soft tissue augmentation, volumetric analysis

1 | 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 & Neiva, 2015; Kothiwale, Rathore, & Panjwani, 2016; Lang & Loe, 1972). Other studies, however, demonstrated that periodontal health was not significantly correlated with the thickness/width of keratinized tissue (Dorfman, Kennedy, & Bird, 1982; Kennedy, Bird, Palcanis, & Dorfman, 1985; Wennstrom & Lindhe, 1983a,b). 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

[†]These authors contributed equally to this work.

tissue might be necessary to overcome the patient's deficiency of obtaining a favourable 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, Acunzo, Cannalire, & Farronato, 2015; Wennstrom, Lindhe, Sinclair, & Thilander, 1987; Zucchelli & Mounssif, 2015). In such a case, recessions could be prevented by two means (Hwang & 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 & Wang, 2006). Traditional autogenous grafts demonstrated clinically predictable outcomes at tooth sites (Cairo, Nieri, & Pagliaro, 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, Schupbach, Ghezzi, Maschera, & Simion, 2012; Schmitt et al., 2016; Thoma, Sancho-Puchades, Ettlin, Hammerle, & Jung, 2012; 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, Rothamel, Herten, Sager, & Becker, 2006; Simion, Rocchietta, Fontana, & Dellavia, 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, Villar, Cochran, Hammerle, & Jung, 2012; Thoma et al., 2010, 2011; Zahedi, Bozon, & Brunel, 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.

Clinical Relevance

Scientific rationale for the study: The gingival thickness is considered to be a critical prognostic factor for gingival recession coverage.

Principal findings: Bovine-derived cross-linked collagen matrice (CCM) resulted in significant gingival thickening in histometric aspects after 12 weeks. Both bovine-derived and porcine-derived CCMs increased the number of rete pegs with narrower and deeper morphological change.

Practical implications: A bovine-derived CCM might potentially serve as a soft tissue substitute for gingival grafting procedures. It is necessary, however, to confirm the results of this experiment in a clinical setting.

2 | MATERIALS AND METHODS

The manuscript was written following the Animal Research: Reporting In Vivo Experiments (ARRIVE) Guidelines Checklist (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010).

2.1 | 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).

2.2 | Experimental animals

Six healthy beagle dogs with the mean age of 12–15 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., 2010, 2011). The animals were housed under constant room temperature (15–20°C) and humidity (>30%).

2.3 | 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.

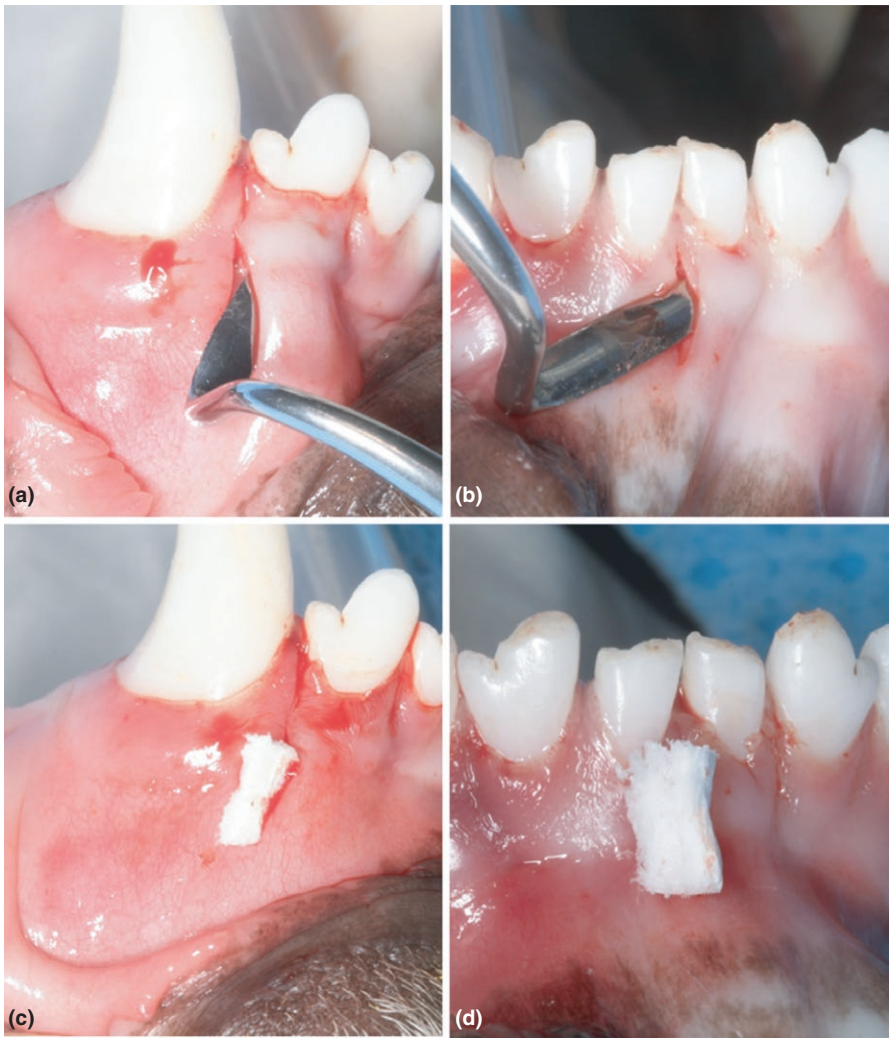

FIGURE 1 Clinical photographs of animal surgery. Pouch formation in bovine CCM (BCCM) group (a) and PCCM group (b). Matrix insertion in BCCM group (c) and PCCM group (d).

2.4 | Surgical intervention

For the surgery, the dogs were anesthetized with intramuscular injection of medetomidine (0.75 mg/kg; Tomidin®; Provet veterinary products Ltd, Istanbul, Turkey) and intravenous injection of alfaxalone (2 mg/kg; Jurox, Rutherford, NSW, Australia). In addition, inhalation anaesthesia with isofluran (Forane®; Choongwae Pharmaceutical, Seoul, Korea) and local infiltration anaesthesia 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 (Figure 1a,b). 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 6 × 4 × 2 mm (length × width × depth; BCCM; Figure 1c) and to 4 × 4 × 2 mm (length × width × depth; PCCM; Figure 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 (20 mg/kg; Yuhan, Seoul, Korea) and non-steroidal anti-inflammatory drugs of meloxicam (0.2 mg/kg; Mobic®; Boehringer Ingelheim, Ingelheim, Germany) administration for 7 days after the surgery. Surgical sites were cleaned daily

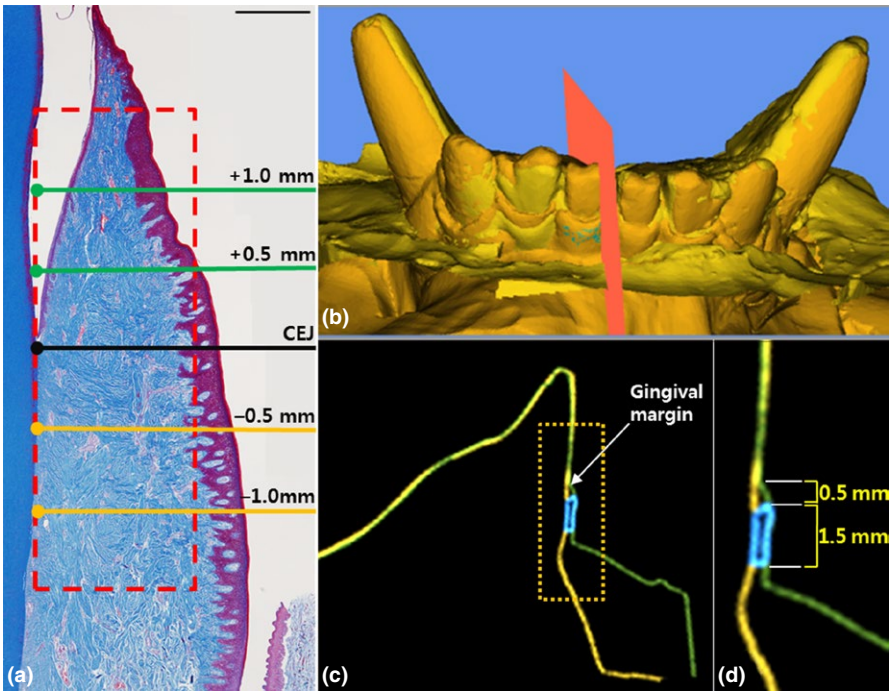

FIGURE 2 Histometric and volumetric measurements. (a) Schematic drawing of histometric analysis. Black bar on the upper right border indicates scale bar of 500 μm in length. Coloured bars with single dot indicate cementoenamel junction (CEJ) level (black), levels above CEJ (green) and levels below CEJ (yellow). Red-dotted rectangle indicates region of interest with 3 mm in height and 1 mm 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 0.5 mm apical to gingival margin to 2.0 mm apical to gingival margin, which is marked in fluorescent blue, for measuring mean distance.

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.

2.5 | Descriptive histology and histometric analyses

The resected specimens were trimmed and embedded in paraffin after decalcification, and the centre-most section was chosen for analyses. The slides were dyed with haematoxylin 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 (Figure 2a):

- Horizontal thickness (T, mm): mean of the data measured at five different levels (1.0 and 0.5 mm above the cementoenamel junction (CEJ), CEJ level, and 0.5 and 1.0 mm 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; 3 mm in height × 1 mm in width).
  - Rete pegs underneath the keratinized epithelium.
  - Rete pegs underneath the sulcular epithelium and junctional epithelium.

2.6 | 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).

2.7 | Volumetric analysis

The resulting STL-files were subsequently analyzed using a software (SMOP; Swissmeda, Zurich, Switzerland) allowing for superimposition with respect to static points (Figure 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 (Figure 2c). The ROI was manually selected as follows: the apico-coronal dimension was determined 0.5 mm apically from the gingival margin and extended 1.5 mm in an apical direction (Figure 2d). The mesio-distal dimension was defined with a clearance of 0.5 mm 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]).

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.

2.9 | 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 $p < 0.05$.

3 | RESULTS

3.1 | 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.

3.2 | 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 fibres and embedded within dense connective tissue with an increased vascularization. None of the PCCM samples showed remnants of the matrix network. (Figure 3).

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: +0.10, +0.13 and +0.15 mm; PCCM: +0.03, +0.05 and +0.07 mm). 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

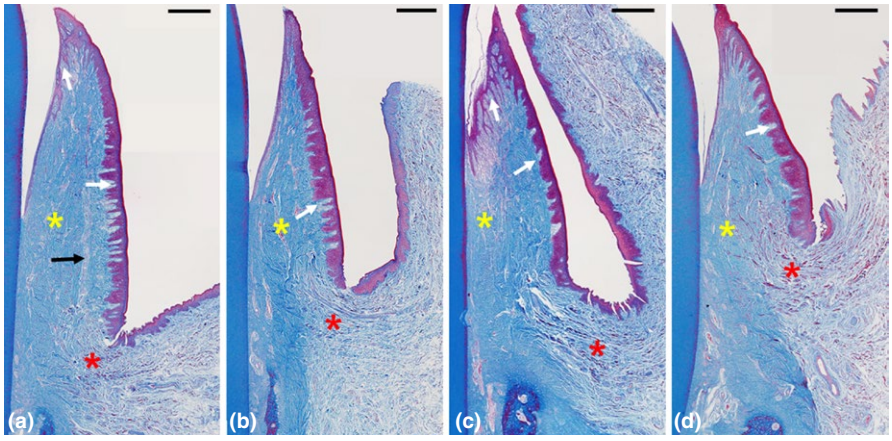

FIGURE 3 Descriptive histology. (a) BCCM group. (b) B-control group. (c) PCCM group. (d) P-control group. Black bar on the upper right border indicates scale bar of 500 μm in length. Coloured asterisks indicate dense connective tissue (yellow) and loose connective tissue (red). Coloured arrows indicate rete pegs (white) and CCM remnant (black).

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

*Significantly different from control group ($p < 0.05$).

underneath the sulcular epithelium and junctional epithelium (BCCM: $p < 0.05$; PCCM: $p > 0.05$) compared to the control groups (Figure 4).

3.4 | 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 ± 0.11 mm in BCCM group and 0.27 ± 0.13 mm in PCCM group. No statistical differences were calculated between groups at any time-point ($p > 0.05$).

4 | 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., 2010, 2011), 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, Bosshardt, & Buser, 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 favourable 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

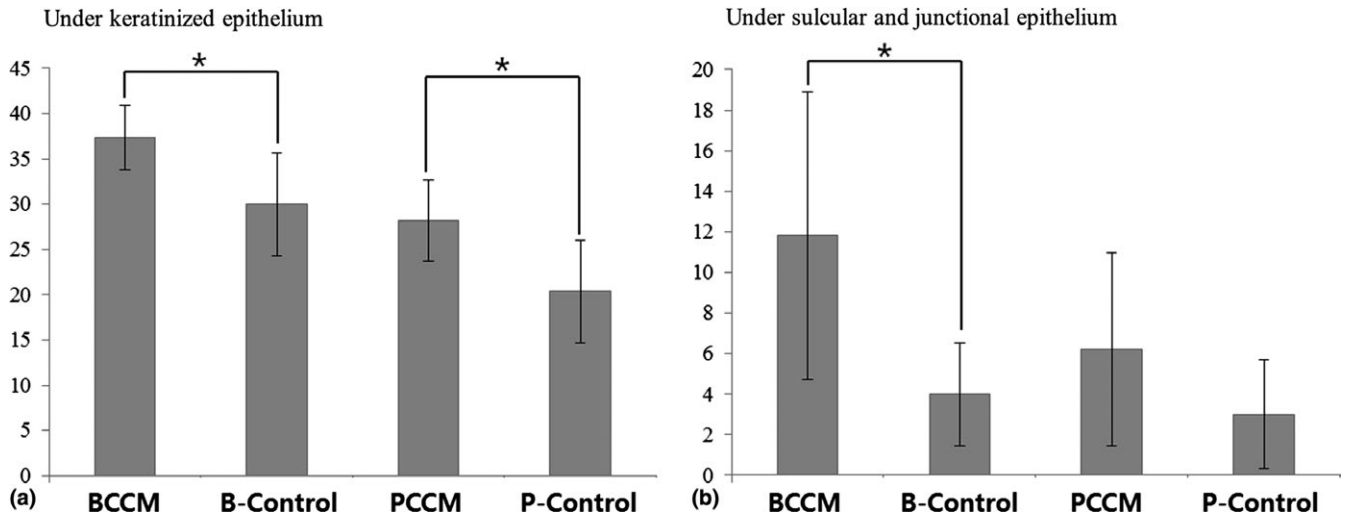

FIGURE 4 Number of rete pegs within the region of interest. *Statistical significance ($p < 0.05$).

TABLE 2 Descriptive statistics of volume change at suture-removal, 4, 8 and 12 weeks compared to pre-surgical state, measured as mean distance (mean ± *SD*; 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

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, Jaasma, & O'Brien, 2009; Rothamel et al., 2005, 2014). 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 remodelling process, involving a maturation with formation of collagen fibres (Ramfjord, Engler, & Hiniker, 1966; Selvig, Bogle, & Claffey, 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 & 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, Villar et al., 2012). 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).

5 | 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.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests.

ORCID

Jae-Kook Cha http://orcid.org/0000-0002-6906-7209
Ui-Won Jung http://orcid.org/0000-0001-6371-4172
Daniel S. Thoma http://orcid.org/0000-0002-1764-7447

REFERENCES

Bornstein, M. M., Bosshardt, D., & Buser, D. (2007). Effect of two different bioabsorbable collagen membranes on guided bone regeneration: A comparative histomorphometric study in the dog mandible. *Journal of Periodontology*, *78*, 1943–1953. https://doi.org/10.1902/jop.2007.070102

Cairo, F., Nieri, M., & Pagliaro, U. (2014). Efficacy of periodontal plastic surgery procedures in the treatment of localized facial gingival recessions. A systematic review. *Journal of Clinical Periodontology*, *41*(Suppl 15), S44–S62. https://doi.org/10.1111/jcpe.12182

Cha, J. K., Joo, M. J., Yoon, S., Lee, J. S., Choi, S. H., & Jung, U. W. (2017). Sequential healing of onlay bone grafts using combining biomaterials

with cross-linked collagen in dogs. *Clinical Oral Implants Research*, *28*, 76–85. https://doi.org/10.1111/clr.12763

Dorfman, H. S., Kennedy, J. E., & Bird, W. C. (1982). Longitudinal evaluation of free autogenous gingival grafts. A four year report. *Journal of Periodontology*, *53*, 349–352. https://doi.org/10.1902/jop.1982.53.6.349

Fickl, S., Fischer, K. R., Jockel-Schneider, Y., Stappert, C. F., Schlagenhauf, U., & Kebschull, M. (2014). Early wound healing and patient morbidity after single-incision vs. trap-door graft harvesting from the palate–a clinical study. *Clinical Oral Investigations*, *18*, 2213–2219. https://doi.org/10.1007/s00784-014-1204-7

Grossman, E. S., & Forbes, M. E. (1990). Studies related to reaction of supporting soft tissue to denture wear: The histological response of vervet monkey oral epithelium to a -80 mmHg vacuum. *Journal of Oral Rehabilitation*, *17*, 587–597.

Haugh, M. G., Jaasma, M. J., & O'Brien, F. J. (2009). The effect of dehydrothermal treatment on the mechanical and structural properties of collagen-GAG scaffolds. *Journal of Biomedical Materials Research Part A*, *89*, 363–369. https://doi.org/10.1002/jbm.a.31955

Hwang, D., & Wang, H. L. (2006). Flap thickness as a predictor of root coverage: A systematic review. *Journal of Periodontology*, *77*, 1625–1634. https://doi.org/10.1902/jop.2006.060107

Jepsen, S., Caton, J. G., Albandar, J. M., Bissada, N. F., Bouchard, P., Cortellini, P., … Yamazaki, K. (2018). Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Clinical Periodontology*, *45*(Suppl 20), S219–S229. https://doi.org/10.1111/jcpe.12951

Jepsen, K., Jepsen, S., Zucchelli, G., Stefanini, M., de Sanctis, M., Baldini, N., … Sanz, M. (2013). Treatment of gingival recession defects with a coronally advanced flap and a xenogeneic collagen matrix: A multicenter randomized clinical trial. *Journal of Clinical Periodontology*, *40*, 82–89. https://doi.org/10.1111/jcpe.12019

Kennedy, J. E., Bird, W. C., Palcanis, K. G., & Dorfman, H. S. (1985). A longitudinal evaluation of varying widths of attached gingiva. *Journal of Clinical Periodontology*, *12*, 667–675.

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Animal research: Reporting in vivo experiments: The ARRIVE guidelines. *British Journal of Pharmacology*, *160*, 1577–1579. https://doi.org/10.1111/j.1476-5381.2010.00872.x

Kim, D. M., & Neiva, R. (2015). Periodontal soft tissue non-root coverage procedures: A systematic review from the AAP regeneration workshop. *Journal of Periodontology*, *86*, S56–S72. https://doi.org/10.1902/jop.2015.130684

Kothiwale, S., Rathore, A., & Panjwani, V. (2016). Enhancing gingival biotype through chorion membrane with innovative step in periodontal pocket therapy. *Cell Tissue Bank*, *17*, 33–38. https://doi.org/10.1007/s10561-015-9524-7

Lang, N. P., & Loe, H. (1972). The relationship between the width of keratinized gingiva and gingival health. *Journal of Periodontology*, *43*, 623–627. https://doi.org/10.1902/jop.1972.43.10.623

Ramfjord, S. P., Engler, W. O., & Hiniker, J. J. (1966). A radioautographic study of healing following simple gingivectomy. II. The connective tissue. *Journal of Periodontology*, *37*, 179–189.

Rasperini, G., Acunzo, R., Cannalire, P., & Farronato, G. (2015). Influence of periodontal biotype on root surface exposure during orthodontic treatment: A preliminary study. *International Journal of Periodontics and Restorative Dentistry*, *35*, 665–675. https://doi.org/10.11607/prd.2239

Rocchietta, I., Schupbach, P., Ghezzi, C., Maschera, E., & Simion, M. (2012). Soft tissue integration of a porcine collagen membrane: An experimental study in pigs. *International Journal of Periodontics and Restorative Dentistry*, *32*, e34–e40.

Rothamel, D., Benner, M., Fienitz, T., Happe, A., Kreppel, M., Nickenig, H. J., & Zoller, J. E. (2014). Biodegradation pattern and tissue integration of native and cross-linked porcine collagen soft tissue augmentation matrices – An experimental study in the rat. *Head and Face Medicine*, *10*, 10. https://doi.org/10.1186/1746-160x-10-10

Rothamel, D., Schwarz, F., Sager, M., Herten, M., Sculean, A., & Becker, J. (2005). Biodegradation of differently cross-linked collagen membranes: An experimental study in the rat. *Clinical Oral Implants Research*, *16*, 369–378. https://doi.org/10.1111/j.1600-0501.2005.01108.x

Schmitt, C. M., Matta, R. E., Moest, T., Humann, J., Gammel, L., Neukam, F. W., & Schlegel, K. A. (2016). Soft tissue volume alterations after connective tissue grafting at teeth: The subepithelial autologous connective tissue graft versus a porcine collagen matrix – A preclinical volumetric analysis. *Journal of Clinical Periodontology*, *43*, 609–617. https://doi.org/10.1111/jcpe.12547

Schwarz, F., Rothamel, D., Herten, M., Sager, M., & Becker, J. (2006). Angiogenesis pattern of native and cross-linked collagen membranes: An immunohistochemical study in the rat. *Clinical Oral Implants Research*, *17*, 403–409. https://doi.org/10.1111/j.1600-0501.2005.01225.x

Schwarz, F., Rothamel, D., Herten, M., Wustefeld, M., Sager, M., Ferrari, D., & Becker, J. (2008). Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: An experimental study in dogs. *Clinical Oral Implants Research*, *19*, 402–415. https://doi.org/10.1111/j.1600-0501.2007.01486.x

Selvig, K. A., Bogle, G., & Claffey, N. M. (1988). Collagen linkage in periodontal connective tissue reattachment. An ultrastructural study in beagle dogs. *Journal of Periodontology*, *59*, 758–768. https://doi.org/10.1902/jop.1988.59.11.758

Simion, M., Rocchietta, I., Fontana, F., & Dellavia, C. (2012). Evaluation of a resorbable collagen matrix infused with rhPDGF-BB in peri-implant soft tissue augmentation: A preliminary report with 3.5 years of observation. *International Journal of Periodontics and Restorative Dentistry*, *32*, 273–282.

Tatakis, D. N., Chambrone, L., Allen, E. P., Langer, B., McGuire, M. K., Richardson, C. R., … Zadeh, H. H. (2015). Periodontal soft tissue root coverage procedures: A consensus report from the AAP Regeneration Workshop. *Journal of Periodontology*, *86*, S52–S55. https://doi.org/10.1902/jop.2015.140376

Thoma, D. S., Hammerle, C. H., Cochran, D. L., Jones, A. A., Gorlach, C., Uebersax, L., … Jung, R. E. (2011). Soft tissue volume augmentation by the use of collagen-based matrices in the dog mandible – a histological analysis. *Journal of Clinical Periodontology*, *38*, 1063–1070. https://doi.org/10.1111/j.1600-051X.2011.01786.x

Thoma, D. S., Jung, R. E., Schneider, D., Cochran, D. L., Ender, A., Jones, A. A., … Hammerle, C. H. (2010). Soft tissue volume augmentation by the use of collagen-based matrices: A volumetric analysis. *Journal of Clinical Periodontology*, *37*, 659–666. https://doi.org/10.1111/j.1600-051X.2010.01581.x

Thoma, D. S., Sancho-Puchades, M., Ettlin, D. A., Hammerle, C. H., & Jung, R. E. (2012). Impact of a collagen matrix on early healing, aesthetics and patient morbidity in oral mucosal wounds – A randomized study in humans. *Journal of Clinical Periodontology*, *39*, 157–165. https://doi.org/10.1111/j.1600-051X.2011.01823.x

Thoma, D. S., Villar, C. C., Cochran, D. L., Hammerle, C. H., & Jung, R. E. (2012). Tissue integration of collagen-based matrices: An experimental study in mice. *Clinical Oral Implants Research*, *23*, 1333–1339. https://doi.org/10.1111/j.1600-0501.2011.02356.x

Vignoletti, F., Nunez, J., de Sanctis, F., Lopez, M., Caffesse, R., & Sanz, M. (2015). Healing of a xenogeneic collagen matrix for keratinized tissue augmentation. *Clinical Oral Implants Research*, *26*, 545–552. https://doi.org/10.1111/clr.12441

Vignoletti, F., Nunez, J., Discepoli, N., De Sanctis, F., Caffesse, R., Munoz, F., … Sanz, M. (2011). Clinical and histological healing of a new collagen matrix in combination with the coronally advanced flap for the

treatment of Miller class-I recession defects: An experimental study in the minipig. *Journal of Clinical Periodontology*, *38*, 847–855. https://doi.org/10.1111/j.1600-051X.2011.01767.x

Wennstrom, J., & Lindhe, J. (1983a). Plaque-induced gingival inflammation in the absence of attached gingiva in dogs. *Journal of Clinical Periodontology*, *10*, 266–276.

Wennstrom, J., & Lindhe, J. (1983b). Role of attached gingiva for maintenance of periodontal health. Healing following excisional and grafting procedures in dogs. *Journal of Clinical Periodontology*, *10*, 206–221.

Wennstrom, J. L., Lindhe, J., Sinclair, F., & Thilander, B. (1987). Some periodontal tissue reactions to orthodontic tooth movement in monkeys. *Journal of Clinical Periodontology*, *14*, 121–129.

Zahedi, S., Bozon, C., & Brunel, G. (1998). A 2-year clinical evaluation of a diphenylphosphorylazide-cross-linked collagen membrane for the treatment of buccal gingival recession. *Journal of Periodontology*, *69*, 975–981. https://doi.org/10.1902/jop.1998.69.9.975

Zucchelli, G., Mele, M., Stefanini, M., Mazzotti, C., Marzadori, M., Montebugnoli, L., & de Sanctis, M. (2010). Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: A comparative randomized-controlled clinical trial. *Journal of Clinical Periodontology*, *37*, 728–738. https://doi.org/10.1111/j.1600-051X.2010.01550.x

Zucchelli, G., & Mounssif, I. (2015). Periodontal plastic surgery. *Periodontology 2000*, *68*, 333–368. https://doi.org/10.1111/prd.12059

How to cite this article: Song YW, Kim S, Waller T, Cha J-K, et al. Soft tissue substitutes to increase gingival thickness: Histologic and volumetric analyses in dogs. *J Clin Periodontol*. 2019;46:96–104. https://doi.org/10.1111/jcpe.13034