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Pre-clinical evaluation of the effect of a volume-stable collagen matrix on periodontal regeneration in two-wall intrabony defects

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Abstract

Aim: To histologically evaluate the effect of a new collagen matrix on periodontal regeneration.

Materials and Methods: Two-wall intrabony defects were surgically created bilaterally distally to the maxillary first and third pre-molars in beagle dogs. The defects were randomly allocated to open flap debridement either with (test) or without (control) a volume-stable collagen matrix (VCMX). After 12 weeks, the dogs were euthanized, and the specimens histologically processed. Descriptive, histomorphometrical (vertical gain of periodontal tissues) and statistical analyses were then performed.

Results: Healing was uneventful in most cases. Residual VCMX was still present and showed integration into new bone, new periodontal ligament, connective tissue and, in some specimens, into new cementum. Periodontal regeneration occurred to a varying extent in both groups. New continuous cementum and new bone formation were statistically significantly greater in the test group (4.12 mm and 3.28 mm, respectively) than in the control group (1.54 mm and 2.47 mm, respectively) (p = .009 and p = .037, respectively). The junctional epithelium was longer in the control group (2.21 mm) than in the test group (1.49 mm, p = .16).

Conclusion: The present results have for the first time provided histologic evidence for the potential of this novel VCMX to facilitate periodontal regeneration thus warranting further pre-clinical and clinical testing.

KEYWORDS
biomaterial, histology, intrabony defect, periodontal regeneration, volume-stable collagen matrix

1 | INTRODUCTION

The rationale to integrate regenerative/reconstructive protocols in the overall treatment concept of periodontal defects is supported by findings from clinical studies showing in general greater clinical improvements when compared to conventional treatments (e.g. access flap surgery) (Avila-Ortiz et al., 2015; Cortellini & Tonetti, 2015; Kao et al., 2015; Sanz et al., 2015; Miron et al., 2016). Furthermore, since regenerative periodontal surgery is a non-resective approach, it may also offer superior aesthetic outcomes as compared to conventional or pocket resective protocols.

Over the last decades, a plethora of clinical protocols including the use of various surgical techniques in conjunction with root surface demineralization, implantation of bone grafts/bone substitutes, guided tissue regeneration (GTR), growth and differentiation factors, enamel matrix derivative (EMD) or various combinations thereof has been shown to enhance periodontal regeneration and to improve the clinical outcomes in intrabony and in class II furcation defects (Sculean et al., 2008; Stavropoulos & Wikesjo, 2012; Avila-Ortiz et al., 2015; Cortellini & Tonetti, 2015; Kao et al., 2015; Sanz et al., 2015; Sculean et al., 2015; Miron et al., 2016; Castro et al., 2017).

Findings from pre-clinical and clinical studies have shown that, from a biologic point of view, the following factors are of pivotal importance for obtaining periodontal regeneration: (a) wound stability to allow undisturbed blood clot adhesion and maturation on the instrumented root surface, (b) space provision to enable formation and maturation of periodontal tissues, and (c) uneventful healing (e.g. without bacterial infection) to support formation and maturation of newly formed tissues (Avila-Ortiz et al., 2015; Cortellini & Tonetti, 2015; Kao et al., 2015; Sanz et al., 2015; Sculean et al., 2015; Susin et al., 2015). Therefore, treatment concepts aiming to provide a clinical benefit should be based on a sound biologic rationale incorporating not only the use of regenerative materials, but also taking into consideration the host's innate healing potential. The decision for selecting the appropriate regenerative material or various combinations is made after careful evaluation of defect anatomy (e.g. non-contained or contained defects) in order to ensure space provision and wound stability.

Since space provision and wound stability have been shown to decisively influence periodontal wound healing and regeneration, novel biomaterials should possess the capacity to stabilize the blood clot and prevent flap collapse thus maintaining the space needed for the regeneration process. A stable blood clot may not only maintain a high concentration of autogenous growth factors in the wound but would also restrict epithelium proliferation. Consequently, formation of new cementum, periodontal ligament (PDL) and bone may occur and would translate clinically into probing depth reduction, gain of clinical attachment level and bone fill (Susin et al., 2015).

Different collagen-based biomaterials are available on the market to replace subepithelial connective tissue and free gingival grafts harvested from the palate (Zuhr et al., 2014). One of this biomaterials is a novel volume-stable collagen matrix (VCMX), which has shown to possess excellent biocompatibility in pre-clinical and human studies (Thoma et al., 2010; Ferrantino et al., 2016; Thoma et al., 2016; Zeltner et al., 2017; Caballe-Serrano et al., 2019; Thoma et al., 2020). Moreover, this biomaterial has demonstrated favourable soft connective tissue integration and promotion of angiogenesis (Thoma et al., 2012; Caballe-Serrano et al., 2019). Due to its biocompatibility and structural configuration (e.g. high porosity and interconnectivity), the material stabilizes the blood clot, while its cross-linked configuration maintains the volume (Mathes et al., 2010).

However, until now, no studies have evaluated the biologic potential of this VCMX to influence periodontal wound healing and regeneration. Therefore, the aim of this study was to investigate the effect of this VCMX to promote periodontal regeneration (formation of new cementum, PDL, bone, and junctional epithelium [JE]) in acute-type two-wall defects in dogs.

Clinical Relevance

Scientific rationale for study: Wound stability is of critical importance for periodontal regeneration. Therefore, we hypothesized that a porous and volume-stable collagen matrix may enhance the regenerative outcome.

Principal findings: In this animal model, periodontal regeneration (i.e. new bone and cementum formation) in intrabony defects was statistically significantly superior with the use of a volume-stable collagen matrix compared to open flap debridement.

Practical implications: This novel volume-stable collagen matrix appears to possess promising properties for enhancing periodontal regeneration. Nevertheless, before its clinical use, further pre-clinical and clinical testing is warranted.

2 | MATERIALS AND METHODS

2.1 | Animals

Eight 18- to 24-month-old beagle dogs, each weighing 12–15 kg, were used. The animals had an intact dentition and a healthy periodontal status. The animals were kept at the animal facility of the Veterinary Faculty of the University of Santiago de Compostela (Lugo, Spain). The dogs were housed under laboratory conditions, at a room temperature of 15–21°C and humidity >30 percentage (%). They had access to tap water ad libitum and laboratory diet.

The current study was conducted in accordance with the European Communities Council Directive 2010/63/EU, approved by the Ethics Committee of the Rof Codina Foundation, Lugo, Spain (02/16/LU-001). In addition, the Guidelines for Animal Research: Reporting In Vivo Experiments (ARRIVE) (Kilkenny et al., 2011) have been included.

2.2 | Study design and sample size

The study was designed as a randomized controlled experiment with one test and one negative control group with a randomized assignment to the groups.

Test group: Open flap debridement (OFD) + VCMX (Geistlich Fibro-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland).
Control group: OFD (alone), negative control.

With eight animals available and four sites per animal, a total of 32 sites were treated. The individual animal was considered as the experimental unit and thus the sample size was eight. This sample size is based on a previous comparable study (Kim et al., 2004).

In order to reduce the risk of bias, the following persons were blinded to the experimental allocation: the animal caregivers, the veterinarian responsible for regular check of animals and the histologist.

2.3 | Surgical procedure

In a first phase, the animals were pre-anesthetized with medetomidine (20 μg/kg/IM, Domtor; Esteve, Barcelona, Spain) and morphine (0.4 mg/kg/IM, Morfina Braun 2%; B. Braun Medical, Barcelona, Spain). The anaesthesia was initiated by propofol (2 mg/kg/IV; Propovet, Abbott Laboratories, Kent, UK) and maintained by inhalation of an O_2 and 2.5%–4% isoflurane mixture (Isobavet, Schering-Plough, Madrid, Spain). A local anaesthesia composed of lidocaine and adrenaline (Anesvet®, Ovejero, Leon, Spain) was used to reduce peri-operative pain and bleeding. After the surgical intervention, atipamezole (50 μg/kg/IM; Antisedan, Esteve) was administered to revert the effects of the medetomidine.

The second and fourth pre-molars of both maxillary quadrants were extracted, and the sites were allowed to heal for 12 weeks. The remaining dentition received oral prophylaxis after the extraction procedure.

In a second phase, the animals were anesthetized like in the first phase. The surgeries were performed by two well-trained periodontists with extensive experience in regenerative periodontal surgery (J.-C.I. and A.S.). Mucoperiosteal flaps were elevated, and acute "box-type" 2-wall intrabony defects of approximately 5 × 5 × 5 millimetres (mm) were surgically created by leaving the palatal bone wall intact (Figure 1a,b). Before bone removal, a coronal reference notch (notch A) was created with a round bur (diameter 1 mm) into the root at the initial alveolar crest. The defects were created at the distal aspects of the first and third pre-molars of both maxillary quadrants by means of rotating and hand instruments. Subsequently, the roots were thoroughly scaled in order to remove the root cementum and PDL. Following root planing, a reference notch (notch B) was created at the apical extension of the defect in the same manner as the coronal notch. The notches served as reference points for the histomorphometric measurements. Thus, all periodontal tissues that would form coronally to the notch are newly formed periodontal tissue. Clinical measurements including the defect depth and width along with intraoral photographs were taken at baseline. The treatment was performed according to the allocated group procedure. Per quadrant a control and a test site were randomly chosen. At the test site a trimmed VCMX, in shape of the defect, was softly compressed into the bony pocket (Figure 1c), whereas the control site was left empty. At the level of the bony defect, the flap with or without VCMX was stabilized by means of a horizontal internal mattress suture. Thereafter, the soft tissue was positioned at the pre-surgical level and the wound was closed (Figure 1d) tension-free by means of monofilament sutures (Ethilon 6-0 blue, Johnson & Johnson Medical GmbH, Ethicon, Norderstedt, Germany).

After the surgeries, pain was controlled with morphine (0.3 mg/kg/IM/6 h) for 24 h and meloxicam (0.1 mg/kg/s.i.d/P.O.; Metacam, Boehringer Ingelheim, Barcelona, Spain) for 4 days. Antibiotics (amoxicillin 22 mg/kg/s.i.d./SC; Amoxoil retard, Syva, Leon, Spain) were administrated for 7 days. The animals were controlled daily for the health symptomatology using standardized score sheets. During the first two postoperative weeks, the oral mucosa and the teeth were disinfected three times a week using gauzes soaked in a chlorhexidine solution (0.12%, Perio-Aid Tratamiento®, Dentaid, Barcelona, Spain). Subsequently, a toothbrush and a chlorhexidine gel (0.2%; Chlorhexidine Bioadhesive Gel, Lacer, Barcelona, Spain) were used three times weekly for plaque control. The dogs were fed

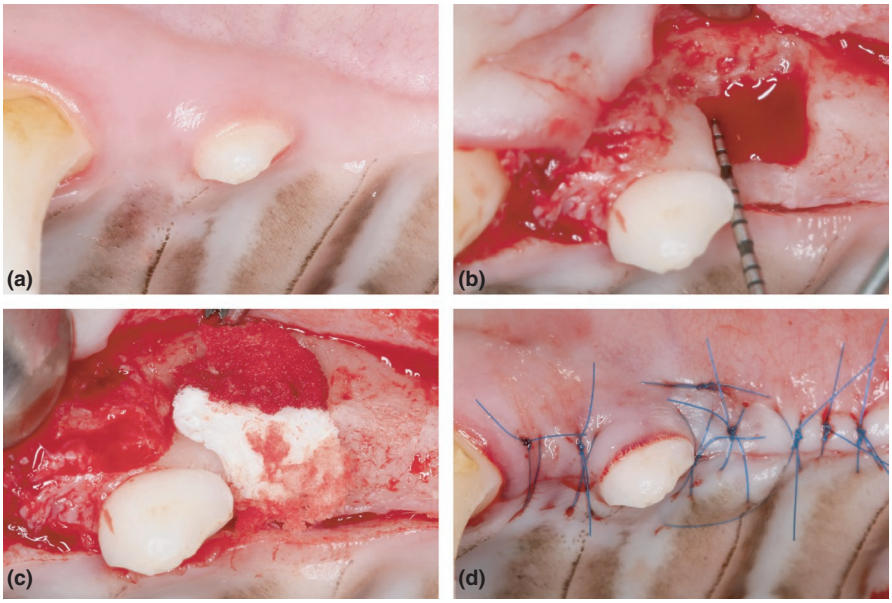

FIGURE 1 Surgical pictures illustrating the procedure in the test group. (a) Pre-surgical, (b) acute-type two-wall defect, (c) after insertion of a trimmed VCMX and (d) after wound closure

a soft-pellet diet for 1 week. The sutures were kept in place during the entire healing time of 12 weeks.

The animals were euthanized 12 weeks after the second phase by sedation with medetomidine (30 μg/kg/IM; Esteve) and subsequently sacrificed with an overdose of sodium pentobarbital (60 mg/kg/IV, Dolethal, Vetoquinol, France).

2.4 | Histological procedures

After euthanization, the maxilla of each animal was removed, and individual bone blocks containing the implanted biomaterials and the surrounding soft and hard tissues were obtained and subsequently fixed in 10% formalin.

Out of 32 defects, 24 (12 test and 12 control sites) were randomly selected for dehydration in an ascending series of ethanol and infiltrated and embedded in methylmethacrylate (MMA). After polymerization, the specimens were sectioned in a mesiodistal plane along their longitudinal axis with a slow-speed diamond saw with a coolant (Varicut® VC-50; Leco, Munich, Germany). Thereafter, the approximately 800-μm-thick ground sections were mounted on Plexiglas slabs and ground to a final thickness of 150 μm (Knuth-Rotor-3; Struers, Rodovre/Copenhagen, Denmark). Finally, the sections were superficially stained with toluidine blue/McNeal combined with basic fuchsin. Furthermore, the remaining 8 defects (4 test and 4 control sites) were decalcified in 10% ethylenediaminetetraacetic acid. They were cut in the same direction as the MMA sections with a microtome set at 8 μm. Staining was done using haematoxylin and eosin. MMA and paraffin sections were produced to perform histomorphometry and descriptive histology. The paraffin sections will be additionally used for future immunohistochemical evaluation. For both processing procedures, photography was performed using a digital camera (AxioCam MRc; Carl Zeiss, Oberkochen, Germany) connected to a light microscope (Axio Imager M2; Carl Zeiss).

2.5 | Histomorphometric analysis

The most-central section (visible apical and coronal notches, presumably central position within the defect area) was chosen for histomorphometric analysis for both, MMA and paraffin sections. Regions of interest were digitalized with a computer connected to a light microscope (Axio Imager M2; Carl Zeiss). Thereafter, all the histomorphometrical landmarks were identified and discussed by two investigators (D.D.B. and J.-C.I.). Since cementum formation was not always continuous from the apical notch to coronal end of the defect, measurements were distinguished between new cementum formation without interruption (new continuous cementum) and discontinuous cementum (new interrupted cementum). The following histomorphometric measurements were performed along the axis through the cementoenamel junction to the apical root using the software Zeiss Efficient Navigation Pro (Zen Pro, Carl Zeiss):

1. Defect height in mm (apical end of notch A to apical end of notch B).
2. Height of new continuous cementum in % and mm (between notch A and notch B).
3. Height of new interrupted cementum in % and mm (between notch A and notch B).
4. New bone height in % and mm (between apical end of notch B and most coronal point of newly formed bone).
5. JE height within gingival sulcus in mm.
6. Connective tissue adhesion height in mm (apical end of the JE to most coronal end of newly formed cementum [i.e. end of new interrupted cementum]).

2.6 | Statistical analysis

Data analyses were performed using Prism v7 (GraphPad Software, La Jolla, CA, USA). To assess the differences between test and control groups, one section per defect was analysed. The measured parameters were calculated as means, standard deviation, medians, minimum, maximum and interquartile ranges. Explorative pre-analyses of the data showed that the effects on the level of the dog were not of importance. Therefore, the differences of means between the two groups were analysed based on the non-parametric Wilcoxon signed-rank test as the distribution of the samples was not normally distributed. Significance was set at $p < .05$. For sample size calculation assuming equal variability and sample size in the two groups, a two-tailed alpha of 0.05, and a power of 80, a minimal number of 10 defects per group were calculated.

3 | RESULTS

3.1 | Clinical findings

During the surgical phase, one tooth of the control group presented an apical pathology. Furthermore, in one first pre-molar site, the sinus was perforated during surgery. After surgery, all animals presented swelling in the operative zone that was resolved within 3–4 days. Thereafter, the healing was uneventful in all dogs without wound dehiscence or other kind of complications encountered.

3.2 | Descriptive histology

All 32 defects were available for descriptive analysis either embedded in MMA (24 defects) or paraffin (eight defects). Processing artefacts were very seldom and never compromised the analysis. Because the sutures were kept in place for the entire healing time, they could be observed in all tissue sections. Around the sutures close to the gingival epithelium, inflammatory cells were present. For sutures deeper in the soft tissues less or no inflammation could be seen, and the extent of inflammation never compromised

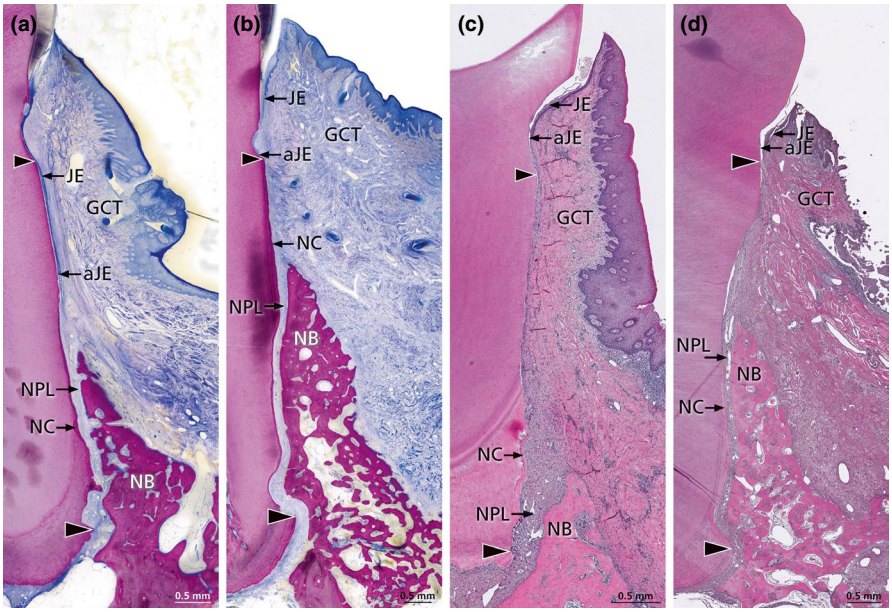

FIGURE 2 Representative overview micrographs of control (a) + test (b) methylmethacrylate sections (a) and control (c) + test (d) paraffin sections. Arrowheads point to the apical ends of coronal and apical root notches, respectively. Methylmethacrylate sections staining: toluidine blue/McNeal + basic fuchsin. Paraffin sections staining: haematoxylin and eosin. aJE, apical end of junctional epithelium; GCT, gingival connective tissue; JE, junctional epithelium; NB, bone; NC, cementum; NPL, periodontal ligament

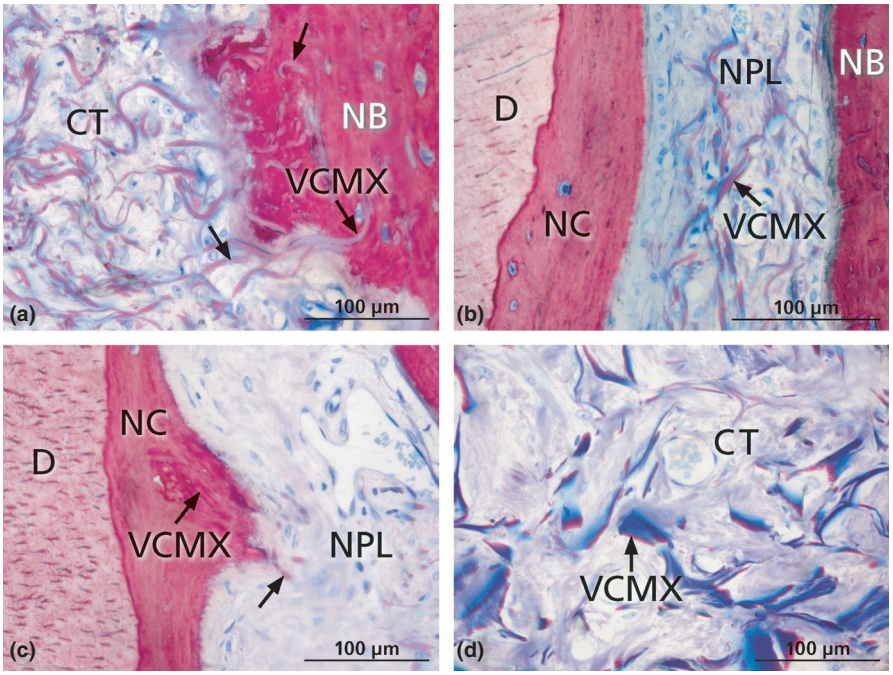

FIGURE 3 Micrographs illustrating integration of collagen matrix residues (VCMX, arrows) into (a) new bone (NB), (b) new periodontal ligament (NPL), (c) new cementum and (d) soft connective tissue (CT) in the coronal defect region. D, dentin. Methylmethacrylate sections staining: toluidine blue/McNeal + basic fuchsin

regeneration in the defect region. In almost all defects, varying amounts of new continuous and interrupted cementum, new bone and new PDL had been formed (Figure 2), and only two teeth in the control group presented no cementum formation at all. Remnants of superficially removed or intact residual cementum were occasionally observed. Furthermore, in all 30 sites with new cementum, the formation was either continuous from the apical end of the defect until it ends coronally (new continuous cementum, eight test and four control sites), or it was interrupted (new interrupted cementum, eight test and ten control sites).

Residual VCMX was still present in all test sites with a varying degree of degradation. In regions of the regenerated alveolar process, the VCMX was always integrated in newly formed bone and PDL (Figure 3a,b, respectively). Moreover, the newly formed PDL had always normal anatomical dimensions, comparable to the pristine tooth site of the same tooth. In addition, the collagen fibres of the newly formed PDL were perpendicularly inserting into the newly formed cementum in nine sites of the test group and five of the control group. Interestingly, in some test sites, residual VCMX could be detected integrated into new cementum (Figure 3c).

Moreover, away from the alveolar process, the residual VCMX coronal to the newly formed bone was infiltrated with blood vessels, fibroblasts and the pores were filled with soft connective tissue (Figure 3d), whereas the apical region of the VCMX was integrated in new bone (Figure 4). In four sites, almost the complete VCMX was

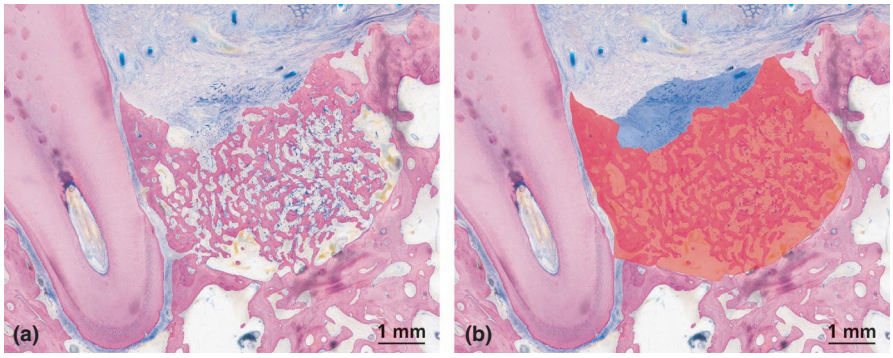

FIGURE 4 (a) and (b) show regeneration of the same test site. (a) Without and (b) with newly formed bone highlighted in red. New soft connective tissue in the coronal defect area is highlighted in blue. Methylmethacrylate sections staining: toluidine blue/McNeal + basic fuchsin

TABLE 1 Histomorphometrical results with mean values and standard deviation (*SD*) for the test and control group

Parameter	Test (mean ± *SD*)	Control (mean ± *SD*)	*p*-value
Defect height			
In mm	5.75 ± 0.74	5.37 ± 0.72	.10
New continuous cementum			
In mm	4.12 ± 1.22	1.54 ± 1.45	.0098
In % of the defect	71.14 ± 17.46	29.09 ± 26.83	.0078
New interrupted cementum			
In mm	5.08 ± 0.94	3.20 ± 2.31	.0391
In % of the defect	89.85 ± 20.92	59.23 ± 43.14	.1055
New bone			
In mm	3.28 ± 0.69	2.47 ± 0.87	.0371
In % of the defect	57.39 ± 11.20	45.39 ± 13.48	.0137
Length of the JE			
In mm	1.49 ± 0.61	2.21 ± 1.43	.1602
Length of the CT-adhesion			
In mm	1.91 ± 1.03	3.36 ± 2.16	.1055

Abbreviations: CT, connective tissue; JE, junctional epithelium; mm, millimetre.

interspersed with new woven bone with still ongoing bone formation (Figure 4). Multinucleated giant cells were never observed.

Two test sites in one animal showed a VCMX with a mass of inflammatory cells, which could account for a contamination during surgery. The surgically already visible apical pathology in one control site was histologically verified.

3.3 | Histomorphometry

Out of 32 defects, nine defects were not suitable for histomorphometrical analysis because of not visible landmarks (three control sites), sinus perforation (one test site), inflammation (two test sites in one animal), apical lesion (one control site) and not removed old cementum on the root surface (two control sites). Therefore, 13 defects of the test (nine MMA and four paraffin) and 10 defects of the control group (eight MMA and two paraffin) were eligible for histomorphometry. The mean values and standard deviations for all sites are presented in Table 1. Minimum and maximum values, median and interquartile range are demonstrated in Table 2. No statistically significant differences were observed between test and control sites for defect height (*p*: .10). The formation of new continuous cementum and bone was analysed in mm and in % of the defect height (Figure 5a,c,d). The absolute and relative heights of the new continuous and interrupted cementum were statistically significant greater in the test compared to the control group (*p*: .0098 and *p*: .0391, respectively). Moreover, absolute and relative values for new bone formation were significantly greater in the test group compared to the control group (*p*: .0371 and *p*: .0137, respectively). Furthermore, the vertical gain in the connective tissue adhesion tended to be greater in the control group than in the test group (Figure 5b) without statistical significance (*p*: .1055). The mean value for the length of the JE was higher in the control group (Figure 5b) but did not reach statistical significance (*p*: .1602).

4 | DISCUSSION

The present study has investigated the regenerative potential of a cross-linked, highly porous VCMX on the healing of acute-type two-wall intrabony defect in dogs. To the best of our knowledge, this is the first study where this novel biomaterial scaffold was tested for periodontal regeneration. Compared to an untreated control defect, the findings of this study demonstrate favourable regenerative outcomes as shown by statistically significantly higher vertical formation of both new continuous and interrupted cementum and new bone when the VCMX was used. Although the height of the JE was smaller in the test group than in the control group, this difference was not statistically significant. Furthermore, the VCMX showed excellent biocompatibility as demonstrated by extensive ingrowth of both bone and soft connective tissue and absence of inflammatory and foreign body giant cells. Moreover, the formation of the new PDL and the orientation of its fibres were not negatively influenced by the VCMX, as demonstrated by more frequent observation of perpendicularly inserting fibres into the newly formed cementum in the test group (nine test sites, five control

TABLE 2 Histomorphometrical results with minimum (Min) and maximum (Max) values, median and interquartile range (IQR) for the test and control group

Parameter	Test (Min/Max)	Control (Min/Max)	Test (median)	Control (median)	Test (IQR)	Control (IQR)
Defect height						
In mm	4.49/6.77	4.46/6.87	5.77	5.25	1.14	0.79
New continuous cementum						
In mm	2.01/5.98	0/4.04	4.09	1.36	1.42	2.43
In % of the defect	40.92/101.83	0/76.82	74.64	25.07	18.71	42.06
New interrupted cementum						
In mm	2.99/6.28	0/6.33	5.21	3.11	1.40	3.35
In % of the defect	52.22/125.79	0/88.28	88.25	56.47	48.01	58.42
New bone						
In mm	1.70/4.57	0.95/3.60	3.32	2.68	0.57	1.35
In % of the defect	29.64/71.83	19.87/68.34	58.56	13.45	47.12	13.55
Length of the JE						
In mm	0.70/2.68	0.77/4.81	1.35	1.47	0.78	1.78
Length of the CT-adhesion						
In mm	0.12/3.75	0.44/6.49	1.81	3.17	1.55	3.33

Abbreviations: CT, connective tissue; JE, junctional epithelium; mm, millimetre.

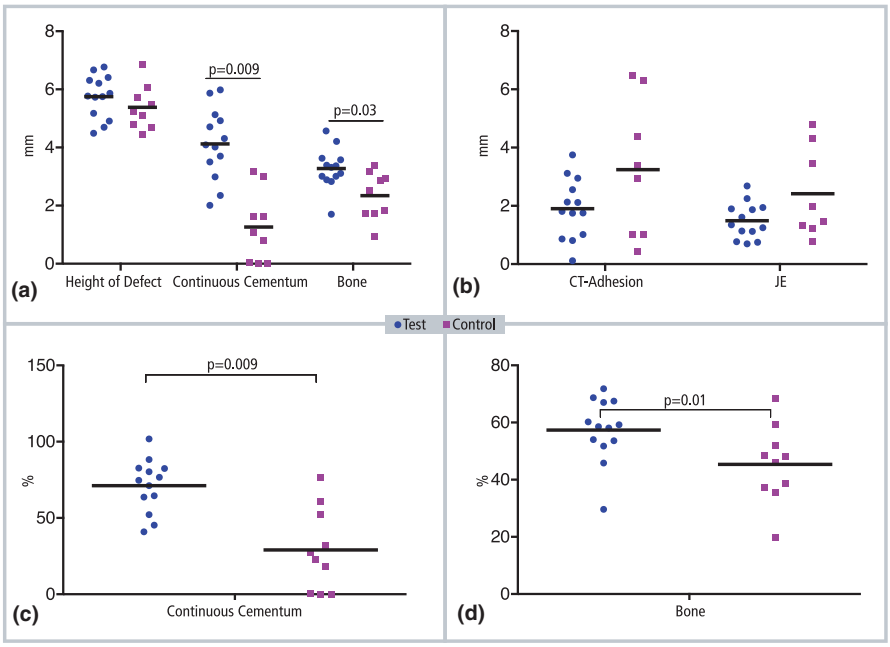

FIGURE 5 Figure of histomorphometric results comparing test and control group. (a) Height of defect, new continuous cementum and new bone in millimetres (mm); (b) connective tissue (CT) adhesion and junctional epithelium (JE) in mm; (c) new continuous cementum in percentage (%); (d) and new bone in % of the defect height

sites). Interestingly, regardless of whether the measurements were done for new continuous or interrupted cementum, the test group yielded statistically significantly superior results compared to the control. To the best of our knowledge, we are not aware of any studies describing the phenomenon of interrupted (i.e. discontinuous) cementum formation on treated root surfaces so far. One possible explanation for the formation of interrupted cementum could be that cementum was not only growing from apical to coronal but also from lateral to central.

Surgeries aiming to restore the periodontal tissues after periodontal diseases are frequently performed and many different types of biomaterials have been used (Sculean et al., 2015). Regenerative/reconstructive periodontal surgeries with some of these biomaterials have shown to provide better clinical outcomes in terms of pocket depth reduction, clinical attachment level gain and hard tissue fill compared to conventional open flap debridement (Kao et al., 2015). Furthermore, in access flap procedures, residual periodontal pockets often persist and resective techniques are associated with

increases in gingival recessions and attachment loss (Rosling et al., 1976; Kaldahl et al., 1996). Biologics like enamel matrix derivative or recombinant human platelet derived growth factor plus β-tricalcium phosphate are comparable with demineralized freeze-dried bone allograft and guided tissue regeneration and superior to open flap debridement (Kao et al., 2015).

A systematic review about periodontal regeneration on intrabony defects in pre-clinical studies has demonstrated new cementum formation of 33%–75% and new bone formation ranging from 12% to 75% of the original defect height with the use of autografts, xenografts, allografts, alloplastic materials, guided tissue regeneration, growth factors and combination therapies (Ivanovic et al., 2014). Guided tissue regeneration for example, one of the best documented methods to obtain periodontal regeneration, showed 66% new cementum and 58% new bone formation. Therefore, treatment of intrabony defects with a VCMX with 71% new continuous cementum, 89% new interrupted cementum and 57% new bone formation represents a promising treatment option with comparable results to well-established treatment modalities.

However, one limitation of the present study is that a positive control with a proven treatment modality to promote periodontal regeneration is missing. Nevertheless, this aspect may be negligible, since there are enough data available in the literature with comparable defect anatomies and healing periods in the same species and comparable defect types and anatomies. Despite the loss of 9 defects for histomorphometrical analysis, the minimal required number (10 defects per group) to reach statistical power was obtained.

The dog is still one of the most well-established animal models for periodontal research (Oz & Puleo, 2011; Kantarci et al., 2015), but translation of results from animal studies to the human situation is problematic because of different anatomical and physiological environments and different healing rates. When interpreting the present findings, it has to be kept in mind that on one hand acute-type defects show obvious morphological, histological and microbiological differences compared to chronic periodontal defects (Selvig, 1994; Donos et al., 2018). Furthermore, depending on their configuration (i.e. contained or non-contained), acute-type defects may show spontaneous healing to a varying extent (Lee et al., 2010; Park et al., 2012) Sculean et al., 2008; Struillou et al., 2010). On the other hand, chronic, ligature-induced defects frequently exhibit substantial morphological differences, making standardization very difficult (Selvig, 1994; Donos et al., 2018).

Furthermore, it has been repeatedly demonstrated that the potential for periodontal regeneration (i.e. formation of periodontal ligament, root cementum and bone) are similar on planed root surfaces previously exposed to periodontal disease to that of surfaces surgically deprived of their attachment apparatus (Isidor et al., 1985; Struillou et al., 2010). Thus, there is evidence to support that despite their shortcomings, acute-type defects are well suitable for pre-clinical studies on periodontal regeneration due also to the possibility of creating standardized defects (Selvig, 1994; Struillou et al., 2010; Donos et al., 2018).

The histological data in this study are clearly showing that the new bone can grow into the VCMX and a new attachment apparatus can be formed with normal anatomical features. Bone ingrowth towards a ridge defect was enhanced in another pre-clinical study using this VCMX (Thoma et al., 2011). Furthermore, in an animal study with gingival recession defects, grafting with a collagen-based biomaterial attained more tissue regeneration characterized by a shorter JE and more vertical new cementum formation compared to a coronally advanced flap alone (Vignoletti et al., 2011).

New concepts to simultaneously regenerate the entire bone-PDL-cementum complex are exploring the effects of stem cells, bioprinting, gene therapy and layered bio-mimetic technologies alone or in combination (Liu et al., 2019). Nowadays, biomaterial design with the use of stem cells and transplantation into patients is a promising field in medicine but difficult to transfer into daily practice (Li et al., 2017). Therefore, biomaterials that act as a scaffold to encourage (or support) the innate regenerative capabilities of the host's own cells from the periodontium could be a valuable option in the field of periodontal reconstructive/regenerative surgery. Endogenous cell homing with the use of biomaterials can be regarded as a more economic, effective and safe method for treating patients (Xu et al., 2019).

The present findings are in line with the results of a study in rat calvaria defects, suggesting that a spongy composition of the collagen barrier membranes may serve like an osteoconductive biomaterial supporting the ingrowth of bony tissue (Kuchler et al., 2018). The tested VCMX did not only stabilize the blood clot but was additionally acting as a scaffold for progenitor cell invasion in soft tissue augmentation (Caballe-Serrano et al., 2019). The cell homing concept of biomaterials in the form of scaffolds could be a possible explanation for the observed VCMX integration in the newly formed periodontal tissues support.

The results of the present study open a wide field for future investigations on the potential use of collagen-based scaffolds in regenerative periodontal therapy. Nevertheless, only one healing period represents a shortcoming of this study. Thus, knowledge about the early wound healing events showing the dynamics of cell migration and invasion into the pores of the VCMX, as well as the sequence of healing and tissue regeneration, is currently missing. Furthermore, the question of combining growth and differentiation factors with the VCMX to further enhance periodontal regeneration should be addressed in future studies. With more biological data from pre-clinical studies available, the last step may include evaluation of efficacy of this biomaterial in human intrabony or even suprabony defects.

In conclusion, the results of the present study have for the first time provided histologic evidence for the potential of this novel VCMX to facilitate periodontal wound healing/regeneration thus warranting further pre-clinical and clinical testing.

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CONFLICT OF INTEREST

This study was financially supported by a grant from Geistlich Pharma AG. The authors report to have no other potential conflict of interest related to this article.

AUTHOR CONTRIBUTIONS

Jean-Claude Imber involved in surgeries, histological procedures, histologic and histomorphometric analysis, interpretation of the results and manuscript writing. Dieter Daniel Bosshardt involved in histological procedures, histologic and histomorphometric analysis, interpretation of the results and manuscript writing. Alexandra Stähli and James Deschner involved in interpretation of the results and manuscript writing. Nikola Saulacic involved in surgeries, histological procedures, histologic and histomorphometric analysis interpretation of the results and manuscript writing. Anton Sculean performed the idea of the study, surgeries, interpretation of the results and manuscript writing.

ETHICAL APPROVAL

The current study was conducted in accordance with the European Communities Council Directive 2010/63/EU, approved by the Ethics Committee of the Rof Codina Foundation, Lugo, Spain (02/16/LU-001).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Avila-Ortiz, G., De Buitrago, J. G., & Reddy, M. S. (2015). Periodontal regeneration - furcation defects: A systematic review from the AAP Regeneration Workshop. *Journal of Periodontology*, *86*(2 Suppl), S108–S130. https://doi.org/10.1902/jop.2015.130677.

Caballe-Serrano, J., Zhang, S., Ferrantino, L., Simion, M., Chappuis, V., & Bosshardt, D. D. (2019). Tissue response to a porous collagen matrix used for soft tissue augmentation. *Materials (Basel)*, *12*(22), 3721. https://doi.org/10.3390/ma12223721.

Castro, A. B., Meschi, N., Temmerman, A., Pinto, N., Lambrechts, P., Teughels, W., & Quirynen, M. (2017). Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: Intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. *Journal of Clinical Periodontology*, *44*(1), 67–82. https://doi.org/10.1111/jcpe.12643.

Cortellini, P., & Tonetti, M. S. (2015). Clinical concepts for regenerative therapy in intrabony defects. *Periodontology 2000*, *68*(1), 282–307. https://doi.org/10.1111/prd.12048.

Donos, N., Park, J. C., Vajgel, A., de Carvalho Farias, B., & Dereka, X. (2018). Description of the periodontal pocket in preclinical models: Limitations and considerations. *Periodontology 2000*, *76*(1), 16–34. https://doi.org/10.1111/prd.12155.

Ferrantino, L., Bosshardt, D., Nevins, M., Santoro, G., Simion, M., & Kim, D. (2016). Tissue integration of a volume-stable collagen matrix in an experimental soft tissue augmentation model. *The International Journal of Periodontics & Restorative Dentistry*, *36*(6), 807–815. https://doi.org/10.11607/prd.3066.

Isidor, F., Karring, T., Nyman, S., & Lindhe, J. (1985). New attachment-reattachment following reconstructive periodontal surgery. *Journal of Clinical Periodontology*, *12*, 728–735. https://doi.org/10.1111/j.1600-051x.1985.tb01398.x.

Ivanovic, A., Nikou, G., Miron, R. J., Nikolidakis, D., & Sculean, A. (2014). Which biomaterials may promote periodontal regeneration in intrabony periodontal defects? A systematic review of preclinical studies. *Quintessence International*, *45*(5), 385–395. https://doi.org/10.3290/j.qi.a31538.

Kaldahl, W. B., Kalkwarf, K. L., Patil, K. D., Molvar, M. P., & Dyer, J. K. (1996). Long-term evaluation of periodontal therapy: II. Incidence of sites breaking down. *Journal of Periodontology*, *67*(2), 103–108. https://doi.org/10.1902/jop.1996.67.2.103.

Kantarci, A., Hasturk, H., & Van Dyke, T. E. (2015). Animal models for periodontal regeneration and peri-implant responses. *Periodontology 2000*, *68*(1), 66–82. https://doi.org/10.1111/prd.12052.

Kao, R. T., Nares, S., & Reynolds, M. A. (2015). Periodontal regeneration - intrabony defects: A systematic review from the AAP Regeneration Workshop. *Journal of Periodontology*, *86*(2 Suppl), S77–S104. https://doi.org/10.1902/jop.2015.130685.

Kilkenny, C.,Browne, W.,Cuthill, I. C.,Emerson, M., Altman, D. G., & National Centre for the Replacement, Refinement and Reduction of Amimals in Research. (2011). Animal research: reporting in vivo experiments-the ARRIVE guidelines. *Journal of Cerebral Blood Flow & Metabolism*, *31*(4), 991–993. https://doi.org/10.1038/jcbfm.2010.220

Kim, C. S., Choi, S. H., Chai, J. K., Cho, K. S., Moon, I. S., Wikesjo, U. M., & Kim, C. K. (2004). Periodontal repair in surgically created intrabony defects in dogs: Influence of the number of bone walls on healing response. *Journal of Periodontology*, *75*(2), 229–235.

Kuchler, U., Rybaczek, T., Dobask, T., Heimel, P., Tangl, S., Klehm, J., Menzel, M., & Gruber, R. (2018). Bone-conditioned medium modulates the osteoconductive properties of collagen membranes in a rat calvaria defect model. *Clinical Oral Implants Research*, *29*(4), 381–388. https://doi.org/10.1111/clr.13133.

Lee, J. S., Wikesjö, U. M., Jung, U. W., Choi, S. H., Pippig, S., Siedler, M., & Kim, C. K. (2010). Periodontal wound healing/regeneration following implantation of recombinant human growth/differentiation factor-5 in a beta-tricalcium phosphate carrier into one-wall intrabony defects in dogs. *Journal of Clinical Periodontology*, *37*(4), 382–389. https://doi.org/10.1111/j.1600-051X.2010.01544.x.

Li, X., He, X. T., Yin, Y., Wu, R. X., Tian, B. M., & Chen, F. M. (2017). Administration of signalling molecules dictates stem cell homing for in situ regeneration. *Journal of Cellular and Molecular Medicine*, *21*(12), 3162–3177. https://doi.org/10.1111/jcmm.13286.

Liu, J., Ruan, J., Weir, M. D., Ren, K. E., Schneider, A., Wang, P., Oates, T. W., Chang, X., & Xu, H. H. K. (2019). Periodontal bone-ligament-cementum regeneration via scaffolds and stem cells. *Cells*, *8*(6), 537. https://doi.org/10.3390/cells8060537.

Mathes, S. H., Wohlwend, L., Uebersax, L., von Mentlen, R., Thoma, D. S., Jung, R. E., Görlach, C., & Graf-Hausner, U. (2010). A bioreactor test system to mimic the biological and mechanical environment of oral

soft tissues and to evaluate substitutes for connective tissue grafts. *Biotechnology and Bioengineering*, *107*(6), 1029–1039. https://doi.org/10.1002/bit.22893.

Miron, R. J., Sculean, A., Cochran, D. L., Froum, S., Zucchelli, G., Nemcovsky, C., & Bosshardt, D. D. (2016). Twenty years of enamel matrix derivative: The past, the present and the future. *Journal of Clinical Periodontology*, *43*(8), 668–683. https://doi.org/10.1111/jcpe.12546.

Oz, H. S., & Puleo, D. A. (2011). Animal models for periodontal disease. *Journal of Biomedicine & Biotechnology*, *2011*, 754857. https://doi.org/10.1155/2011/754857.

Park, J.-C., Wikesjö, U. M. E., Koo, K.-T., Lee, J.-S., Kim, Y.-T., Pippig, S. D., Bastone, P., Kim, C.-S., & Kim, C.-K. (2012). Maturation of alveolar bone following implantation of an rhGDF-5/PLGA composite into 1-wall intra-bony defects in dogs: 24-week histometric observations. *Journal of Clinical Periodontology*, *39*(6), 565–573. https://doi.org/10.1111/j.1600-051X.2012.01868.x.

Rosling, B., Nyman, S., & Lindhe, J. (1976). The effect of systematic plaque control on bone regeneration in infrabony pockets. *Journal of Clinical Periodontology*, *3*(1), 38–53.

Sanz, M., Jepsen, K., Eickholz, P., & Jepsen, S. (2015). Clinical concepts for regenerative therapy in furcations. *Periodontology 2000*, *68*(1), 308–332. https://doi.org/10.1111/prd.12081.

Sculean, A., Nikolidakis, D., Nikou, G., Ivanovic, A., Chapple, I. L., & Stavropoulos, A. (2015). Biomaterials for promoting periodontal regeneration in human intrabony defects: A systematic review. *Periodontology 2000*, *68*(1), 182–216. https://doi.org/10.1111/prd.12086.

Sculean, A., Nikolidakis, D., & Schwarz, F. (2008). Regeneration of periodontal tissues: Combinations of barrier membranes and grafting materials - Biological foundation and preclinical evidence: A systematic review. *Journal of Clinical Periodontology*, *35*(8 Suppl), 106–116. https://doi.org/10.1111/j.1600-051X.2008.01263.x.

Selvig, K. A. (1994). Discussion: Animal models in reconstructive therapy. *Journal of Periodontology*, *65*(12), 1169–1172. https://doi.org/10.1902/jop.1994.65.12.1169.

Stavropoulos, A., & Wikesjo, U. M. (2012). Growth and differentiation factors for periodontal regeneration: A review on factors with clinical testing. *Journal of Periodontal Research*, *47*(5), 545–553. https://doi.org/10.1111/j.1600-0765.2012.01478.x.

Struillou, X., Boutigny, H., Soueidan, A., & Layrolle, P. (2010). Experimental animal models in periodontology: A review. *The Open Dentistry Journal*, *29*, 37–47. https://doi.org/10.2174/1874210601004010037.

Susin, C., Fiorini, T., Lee, J., De Stefano, J. A., Dickinson, D. P., & Wikesjo, U. M. (2015). Wound healing following surgical and regenerative periodontal therapy. *Periodontology 2000*, *68*(1), 83–98. https://doi.org/10.1111/prd.12057.

Thoma, D. S., Gasser, T. J. W., Jung, R. E., & Hammerle, C. H. F. (2020). Randomized controlled clinical trial comparing implant sites augmented with a volume-stable collagen matrix or an autogenous connective tissue graft: 3-year data after insertion of reconstructions. *Journal of Clinical Periodontology*, *47*(5), 630–639. https://doi.org/10.1111/jcpe.13271.

Thoma, D. S., Hämmerle, C. H. F., Cochran, D. L., Jones, A. A., Görlach, C., Uebersax, L., Mathes, S., Graf-Hausner, U., & 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*(11), 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., Görlach, C., Uebersax, L., Graf-Hausner, U., & Hämmerle, C. H. F. (2010). Soft tissue volume augmentation by the use of collagen-based matrices: A volumetric analysis. *Journal of Clinical Periodontology*, *37*(7), 659–666. https://doi.org/10.1111/j.1600-051X.2010.01581.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*(12), 1333–1339. https://doi.org/10.1111/j.1600-0501.2011.02356.x.

Thoma, D. S., Zeltner, M., Hilbe, M., Hammerle, C. H., Husler, J., & Jung, R. E. (2016). Randomized controlled clinical study evaluating effectiveness and safety of a volume-stable collagen matrix compared to autogenous connective tissue grafts for soft tissue augmentation at implant sites. *Journal of Clinical Periodontology*, *43*(10), 874–885. https://doi.org/10.1111/jcpe.12588.

Vignoletti, F., Nuñez, J., Discepoli, N., De Sanctis, F., Caffesse, R., Muñoz, F., Lopez, M., & 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*(9), 847–855. https://doi.org/10.1111/j.1600-051X.2011.01767.x.

Xu, X. Y., Li, X., Wang, J., He, X. T., Sun, H. H., & Chen, F. M. (2019). Concise review: Periodontal tissue regeneration using stem cells: Strategies and translational considerations. *Stem Cells Translational Medicine*, *8*(4), 392–403. https://doi.org/10.1002/sctm.18-0181.

Zeltner, M., Jung, R. E., Hammerle, C. H., Husler, J., & Thoma, D. S. (2017). Randomized controlled clinical study comparing a volume-stable collagen matrix to autogenous connective tissue grafts for soft tissue augmentation at implant sites: Linear volumetric soft tissue changes up to 3 months. *Journal of Clinical Periodontology*, *44*(4), 446–453. https://doi.org/10.1111/jcpe.12697.

Zuhr, O., Baumer, D., & Hurzeler, M. (2014). The addition of soft tissue replacement grafts in plastic periodontal and implant surgery: Critical elements in design and execution. *Journal of Clinical Periodontology*, *41*(Suppl 15), S123–S142. https://doi.org/10.1111/jcpe.12185.

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