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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

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Abstract

Aim: To describe the histological and clinical outcomes of the use of a xenogeneic collagen matrix (CM) in combination with the coronally advanced flap (CAF) in the treatment of localized Miller class-I gingival recessions.

Material and Methods: Gingival recession defects were surgically created on 12 minipigs. The defects were randomly treated with either the CAF procedure and the interposition of a CM (test) or the CAF alone (control). Clinical and histological outcomes at 1, 4 and 12 weeks were evaluated.

Results: Histometrically, in the test group, there was a shorter junctional epithelial dimension [2.26 (SD 0.23) mm] compared with the control [2.79 (SD 0.77) mm]. On the contrary, the amount of newly formed cementum was larger in the test group [1.08 (SD 0.41) mm] than in the control group [0.75 (SD 0.25) mm], although the differences were not statistically significant.

Conclusions: Both techniques rendered similar clinical outcomes, achieving complete root coverage at the end of the study. Nevertheless, the CM graft attained more tissue regeneration, characterized by a shorter epithelium and a larger new cementum formation. The use of a xenogeneic CM resulted in the incorporation of the xenograft within the adjacent host connective tissues in the absence of significant inflammation.

Key words: collagen matrix; histometry; localized gingival recessions; mini-pigs; xenograft

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The treatment of localized gingival recessions is a common therapeutic procedure in dental practice. The displacement of the gingival margin apical to the cemento-enamel junction results in root exposure to the oral cavity, which may cause aesthetic concern, root sensitivity or progressive recession. Several surgical procedures have been proposed with the goal of covering these recession defects (Roccuzzo et al. 2002). A recent systematic review (Chambrone et al. 2010) reported that the mean percentage of root coverage among the different surgical techniques ranged between 35% and 97%, the subepithelial connective tissue graft (CTG) being the surgical approach that provided the best outcomes. This technique requires the use of an autograft, most frequently harvested from the patient's palatal mucosa. In order to avoid this second surgical site, the coronally advanced flap (CAF) has been indicated in the treatment of Miller class-I and class-II recession defects. The CAF procedure consists of the coronal displacement of the soft tissue margin over the exposed root surface. Since the first presentation of the technique (Allen & Miller 1989), different modifications of this surgical procedure have been proposed to enhance the predictability of root coverage (de Sanctis & Zucchelli 2007). Recently, (Cairo et al. 2008) evaluated systematically the literature on the efficacy of this surgical procedure. The authors reported that both the use of enamel matrix derivatives and the placement of a CTG in combination with CAF enhanced the probability of achieving complete root coverage.

In spite of the extensive clinical evaluation of these surgical procedures, there is limited histological evidence on the type of healing that occurs (Pasquinelli 1995, Goldstein et al. 2001, Cummings et al. 2005, McGuire et al. 2009). All these studies described a healing characterized by a combination of new attachment in the apical portion of the defect and repair in the coronal part, through a long junctional epithelium and connective tissue adhesion.

In order to avoid the need for harvesting an autograft, different techniques and materials, such as allografts, xenografts or soft tissue engineered grafts, have been proposed as soft tissue substitutes. Among them, the allogenic acellular dermal matrix graft (Alloderm®, Life Cell Corporation, The Woodlands, TX, USA) has been the most frequently used (Thoma et al. 2009). Recently, a xenogeneic collagen matrix (CM) of porcine origin (Geistlich Mucograft®, Geistlich Pharma AG, Wolhusen, Switzerland) has been developed and its qualitative properties and safety have been evaluated following the ISO 14971 and ISO 10993-1 guidelines. The clinical efficacy of this new CM was assessed to build up a clinically sufficient width of newly formed gingiva/mucosa as well as to cover Miller class-I and class-II recessions (Sanz et al. 2009, McGuire & Scheyer 2010).

In spite of the promising clinical results, there is no information in the literature on the type of histological healing that occurs when this CM is interposed between the root surface and the flap in the treatment of localized gingival recessions. It is, therefore, the objective of this investigation to describe the histological and clinical outcomes of the use of a xenogeneic CM in combination with the CAF procedure in the treatment of gingival Miller class-I recessions.

Material and Methods

Animals

Twelve Gottingen 24-month-old female minipigs were included in the present animal experiment. The animals weighed between 35 and 40 kg and were kept in a purpose-designed centre for large experimental animals at the Veterinary Teaching Hospital Rof Codina in Lugo, Spain. The Regional Ethics Committee for Animal Research at the University of Lugo approved the protocol. The animals were kept on a soft diet and subjected to oral hygiene by mechanical cleaning once every 3 weeks during the experimental study.

Study device

The xenogeneic CM of porcine origin (Geistlich Mucograft®, Geistlich Pharma AG) is a class-III medical device according to the Medical Device Directive 93/42. This three-dimensional matrix consists of two functional structures: a compact layer consisting of collagen fibres in a dense arrangement and a thick porous scaffold. This spongy scaffold provides a space that favours the formation of a blood clot and the in-growth of tissue from adjacent sites.

Surgeries

All surgical procedures were performed under general anaesthesia and sterile conditions in an operating room using a mixture of isoflurane 2 l/h (IsobaVet, Intervet, Madrid, Spain) and oxygen with a mechanical respirator throughout the surgical procedure. The animals had been sedated previously with a cocktail containing 80 μg/kg of Medetomidine (Domtor, Pfizer, Madrid, Spain), 20 mg/kg of Butorfanol (Torbugesics, Fort Dodge, Gerona, Spain) and 1 mg/kg of Atropine Sulphate (Atropinas, Instituto Farmaceutico FAS, Burgos, Spain), then intubated, anaesthetized and monitored with an EKG during the surgery.

Surgery 1: creation of the recession defect

The creation of the recession defect was performed according to a modification of a method described previously by Nuñez et al (2009). Once anaesthetized, two vertical releasing incisions were performed on the mesio-buccal aspect of the mandibular and maxillary PI or PIII. A muco-periosteal full-thickness flap was then elevated to expose the buccal alveolar bone. A dehiscence defect 5 mm in depth and 4 mm in width was created apical to the cemento-enamel junction using bone chisels (Fig. 1a). The flap was then apically repositioned and sutured, leaving the root surface exposed (Fig. 1b).

Surgery 2: root coverage procedures

The second set of surgeries was carried out 1 month after Surgery 1 (Fig. 1d,e). Once the animals were anaesthetized, two horizontal bevelled incisions were drawn mesial and distal to the recession, 1–2 mm coronal to the most apical portion of the recession. Vertical incisions were then made through the alveolar mucosa. The flap was elevated following a split-full-split thickness approach in a corono-apical direction. A notch was then prepared with a diamond round bur on the root surface at the level of the bone crest, under abundant irrigation. The mesial and distal papillae below the horizontal incisions were de-epithelized and the root surface was mechanically debrided. At this time point, randomization was performed through a computer-originated list and the recessions were assigned to either receive the CAF plus CM (test sites) or CAF alone (control sites).

In the test sites, the CM was trimmed to adapt to the recipient bed extending 2–3 mm apical to the buccal bone and thus avoiding the suture lines. The CM was secured in place with t-mattress resorbable sutures (Vicryl, 4.0) (Fig. 1d). The flap was then coronally advanced and stabilized using marginal direct sutures on the vertical releasing incisions and a final sling suture to adapt the flap to the neck of the tooth at the inter-dental papillae (Fig 1e).

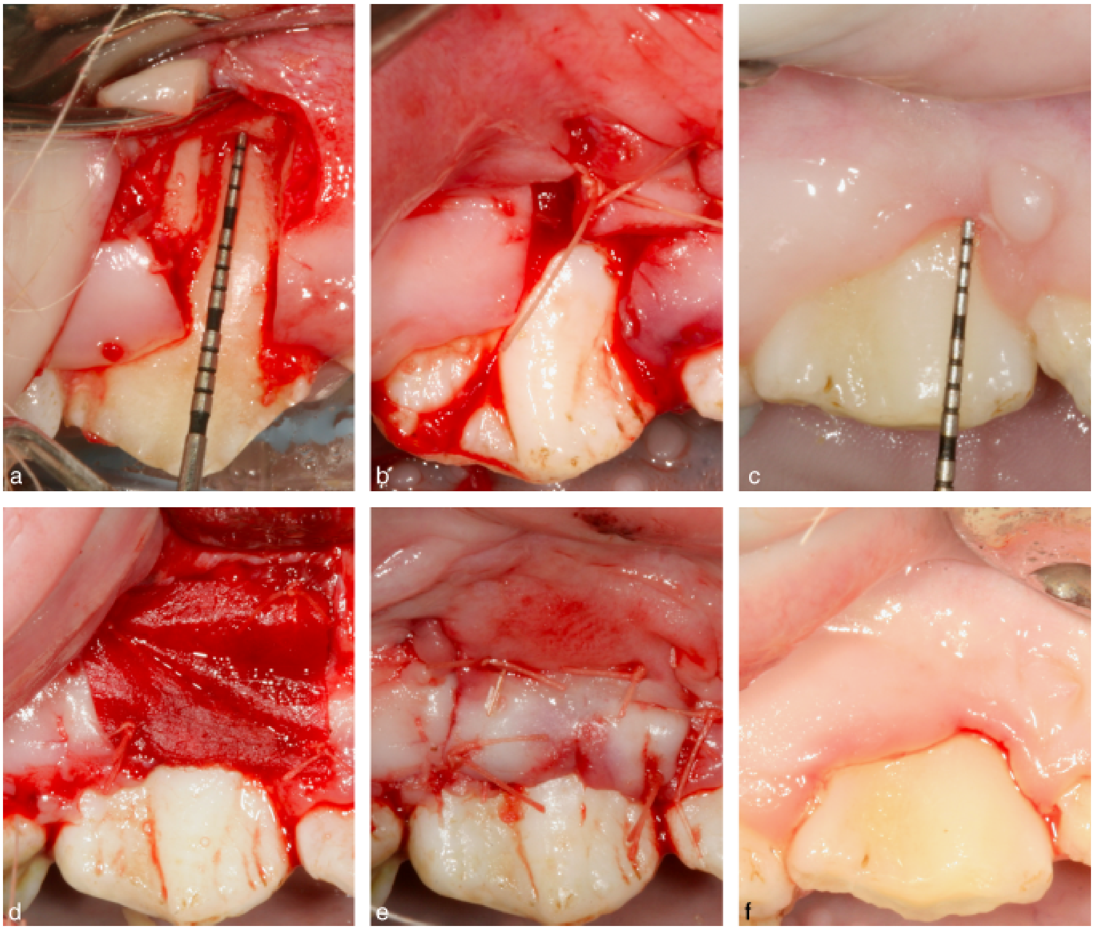

Fig. 1. Surgery 1. Creation of the mucogingival recessions: a dehiscence defect 5 mm in depth (a) and 4 mm in width was created apical to the cemento-enamel junction. The flap was apically repositioned and sutured (b). Surgery 2. (c) Clinical situation after 1 month of healing. (d, e) The coronally advanced flap procedure was performed. In the test group, the xenogenic collagen matrix was interposed and sutured between the root surface and the flap (d). (f) Clinical photograph after 3 months of healing.

Experimental design

The present study was designed as a randomized-controlled experimental study using 12 Gottingen 24-month-old female minipigs. Animals were divided into three groups to allow the evaluation of three healing periods, at 1, 4 and 12 weeks after treatment. Each group included four animals that provided four study sites each, with two test and two controls.

Histological processing

The animals were sedated and euthanized through an overdose of sodium pentobarbital (Dolethal©, Vétoquinol, France). The upper and lower jaws were removed and immersed in 10% neutral-buffered formalin for 1 week (Exakt, Norderstedt, Germany). Buccolingual tissue blocks, containing teeth and surrounding soft and hard tissues, were obtained using a band saw system (Exakt, Norderstedt, Germany). Specimens were decalcified for 24–36 h (depending on the thickness) by immersing in a commercial solution containing EDTA di-sodium salt (volume ratio specimen/decalcifier 1:100) (Osteodec, Bio-Optica, Milano, Italy) with an acid buffer. Once reconditioned, using three steps of PBS at pH 7.4, the specimens were embedded in paraffin. Sections of 5 μm thickness were stained with haematoxylin and eosin (HE) and Mallory's connective tissue stain for their evaluation by light microscopic examination.

Histological evaluation

Serial sections from the furcation area to the mesial aspect of the tooth root were obtained, with the two central sections chosen for histological evaluation, resulting in four decalcified sections per group in each animal. All the histometric measurements were carried out blindly by two calibrated investigators (J. N., F. V.) in a Nikon Eclipse Ti microscope (Nikon, Heidelberg, Germany) equipped with an image analysis software (Q-500 MC, Nikon).

In each section, the following landmarks were identified (Fig. 2):

- *M*: the most coronal marginal mucosa,
- *aJE*: the most apical extent of the junctional epithelium,
- *cC*: the most coronal extent of the cementum,
- *aN*: the most apical portion of the notch,
- *Bc*: the most coronal level of the bone crest.

Furthermore, the tissue width was measured at the mid-central portion of the junctional epithelium.

Clinical evaluation

Before the experimental surgeries and before sacrifice, measurements of probing pocket depths (not shown), gingival recession from the CEJ to the gingival margin and keratinized tissue width were recorded using an NC15 periodontal probe (PCP-UNC 15, Hu-Friedy Manufacturing Co., Chicago, IL, USA).

Statistical analysis

The means and standard deviations for each histological parameter were calculated for both experimental and control groups, the animal being the experimental unit for all the comparisons ($n = 4$). Because of the small sample size, the differences between groups were analysed using the Mann–Whitney test for both the histological and the clinical outcome variables.

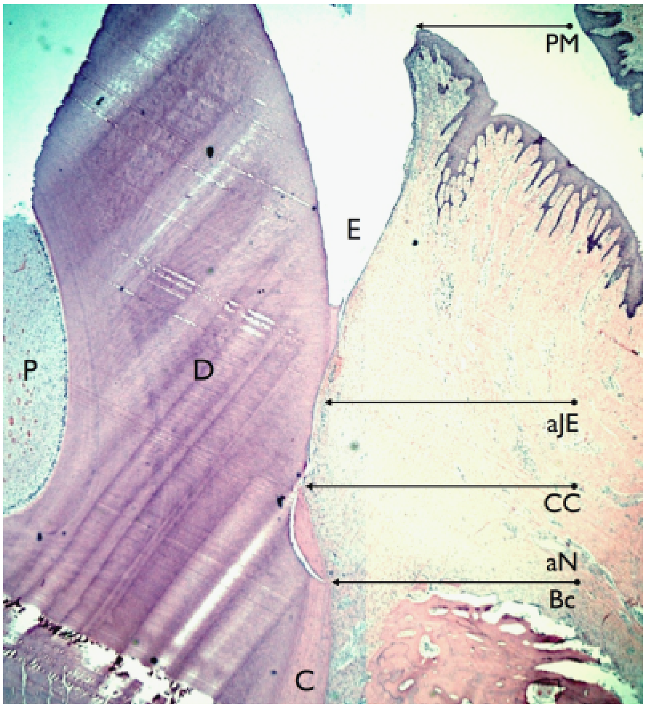

Fig. 2. Cross-section of the buccal dento-gingival region. Landmarks utilized for histometric measurements. PM, most coronal marginal mucosa; aJE, most apical extent of the junctional epithelium; cC, most cornal extent of cementum formation; An, most apical portion of the notch; Bc, most coronal level of the bone crest (Bc and aN are at the same level in the present histological section). Haematoxylin and eosin (HE) stain. Original magnification × 20. P, pulp tissue; D, dentin; C, root cementum; E, enamel.

Results

The post-operative healing in all experimental surgeries was uneventful and the animals demonstrated good behaviour, as shown by their eating and drinking ad libitum. The clinical results are depicted in Table 1. One site in one animal did not achieve complete root coverage in the test group, whereas all sites in the control group presented 100% coverage of the previously exposed root. Overall, in both groups, the experimental surgeries resulted in a statistically significant reduction in the depth of the recessions, although the differences between test and control surgeries were not significant.

Table 1. Clinical measurements of keratinized tissue width (mean (SD) mm) and recession (REC) length (mean (SD) mm)

Healing period	Keratinized tissue		Recession	
	Test	Control	Test	Control
Baseline	2.38 (0.71)*	2.42 (0.87)	1.79 (0.58)*	1.90 (0.40)¶
1month	3.25 (0.29)*^	3.00 (1.22)	1.50 (0.41)^	1.00 (0.41)¶
3 months	2.50 (0.41)^	3.38 (0.25)	0.13 (0.25)*^	0.00 (0.00)¶

Intra-group Diff.St.Sig.*^$p < 0.05$. Intra-group Diff.St.Sig.*^¶$p < 0.001$.

Histological observations: 1 week of healing

At 1 week, the CM was identified in the underlying connective tissue surrounded by an inflammatory infiltrate mainly composed of polymorphonuclear leucocytes. Within this inflammatory infiltrate, immature fibroblast-like cells and vascular buds were identified. An initial junctional epithelium was also established. The alveolar crest showed marginal remodelling with the presence of osteoclasts both at the periosteal and at the periodontal side. In two instances, the CM was exposed, and scattered patches of immature epithelium were identified, but without depicting a continuous layer (Fig. 3b,c). These epithelial nests were intermixed with an inflammatory exudate covering the exposed matrix. The oral epithelium showed areas of apical migration between the flap and the surface of the CM. Within this open wound-healing environment, the body of the CM showed a high density of vascular channels and immature fibroblast-like cells. No multinucleated giant-cells or macrophages were identified under the light microscope, thus excluding any foreign body reaction. The marginal bone crest showed signs of resorption. The control specimens showed a more regular pattern of healing. The oral epithelium usually reached the tooth surface and the junctional epithelium was already forming. The inflammatory infiltrate was usually limited to the supra-crestal tissues in the vicinity of the root surface. The bone crest showed signs of remodelling, whereas the periodontal ligament space did not show any significant change.

1 month of healing

The test group demonstrated almost complete epithelial healing by the establishment of a well-defined junctional epithelium. A small inflammatory infiltrate was confined in the marginal subcrevicular area, whereas the rest of the supra-crestal connective tissue compartment showed a healthy fibrous structure with dispersed fibroblasts and vascular

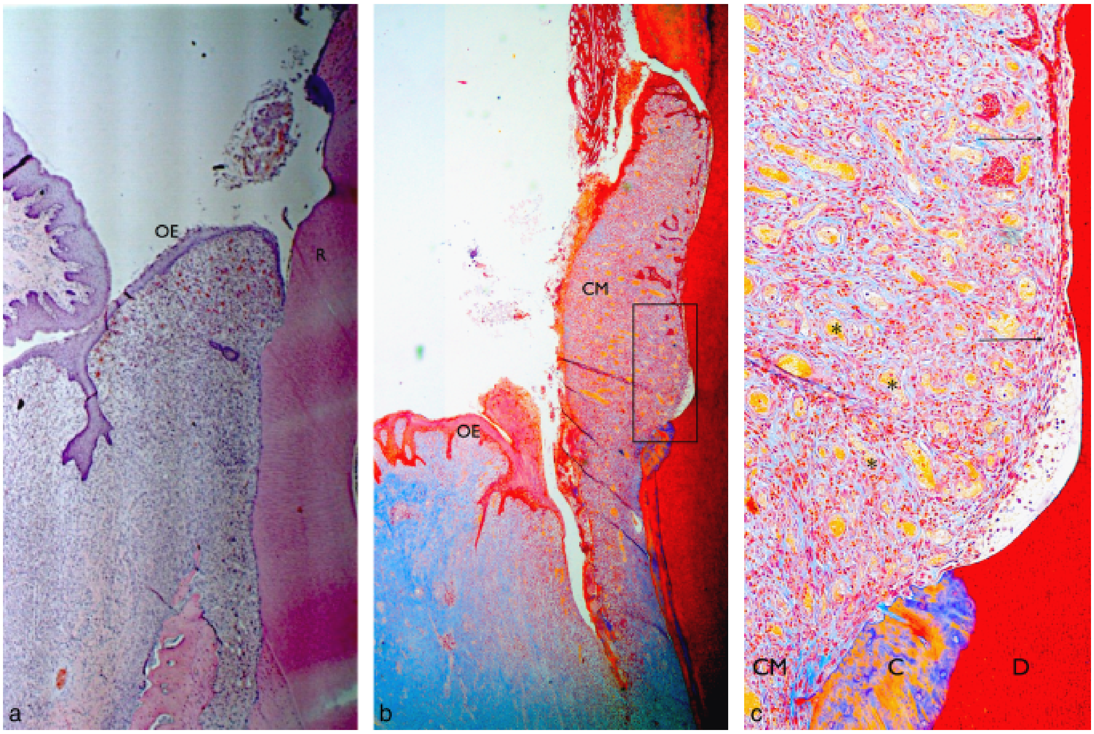

Fig. 3. Cross-section of the buccal dento-gingival region representing the test group after 1 week of healing. (a) An immature oral epithelium (OE) started to migrate apically on the root surface (R). (b) The flap receded and the xenogenic collagen matrix (CM) was exposed. The OE showed areas of apical migration between the inner aspect of the flap and the surface of the collagen matrix. (b) Mallory's connective tissue stain. Original magnification $\times$ 20. (c) Detail of (b). Note the high density of vascular channels (*). Patches of immature epithelium (arrows) are present on the root surface close to the notch. D, dentin; C, cementum. Mallory's connective tissue stain. Original magnification $\times$ 100.

buds. The CM could not be differentiated from the rest of the supracrestal connective tissue. Evidence of new cementum formation with the presence of inserting collagen fibres was observed (Fig. 4 a,b).

The control specimens showed a similar histological picture, except for the presence of a higher inflammatory infiltrate under the junctional epithelium, which, in some specimens, reached the notch area. The epithelium reached the apical end of the notch in most of the sections, demonstrating an irregular pattern with deep rete-pegs. The bone surface was lined with osteoblasts both on the periodontal and on the periosteal sides of the crest (Fig. 4c).

3 months of healing

The specimens in the test group reached complete healing, demonstrating adequate maturation and fibre orientation. The junctional epithelium was fully established, whereas the connective tissue compartment at this level showed the formation of a fibre attachment apparatus to the newly developed cementum in the area of the notch (Fig. 5a,b).

The control specimens showed a similar pattern of healing, with the exception that the established junctional epithelium usually reached the vicinity of the notch, although some degree of new-cementum deposition was frequently found (Fig. 5c).

Histometric analysis

The results from the histometric evaluation are depicted in Table 2. Only the analysis from 1- and 3-month specimens was carried out, as in the 1-week specimens, the landmarks could not be easily identified.

Length of the junctional epithelium

The distance between the coronal marginal mucosa and the most apical extent of the junctional epithelium (M – aJE) was similar between the test and the control groups in the 1-month specimens. At 3 months, the control group showed a further apical proliferation of the junctional epithelium, while in the test group, the epithelial length remained stable. This resulted in a shorter junctional epithelium in the test group compared with the control group after 3 months, although the differences were not statistically significant.

Length of the connective tissue adhesion

The distance between the apical extent of the junctional epithelium and the most coronal extent of cementum (cC – aJE) was small and similar in the test and control groups. The difference between the test and control groups was statistically significant at 1 month whereas no differences were observed between groups at the end of the study. However, a tendency towards a longer connective tissue adhesion was observed in the control group at both healing time points.

Length of new cementum

The distance from the most apical end of the notch to the most coronal extent of the cementum (aN – cC) was very similar between the test and the control

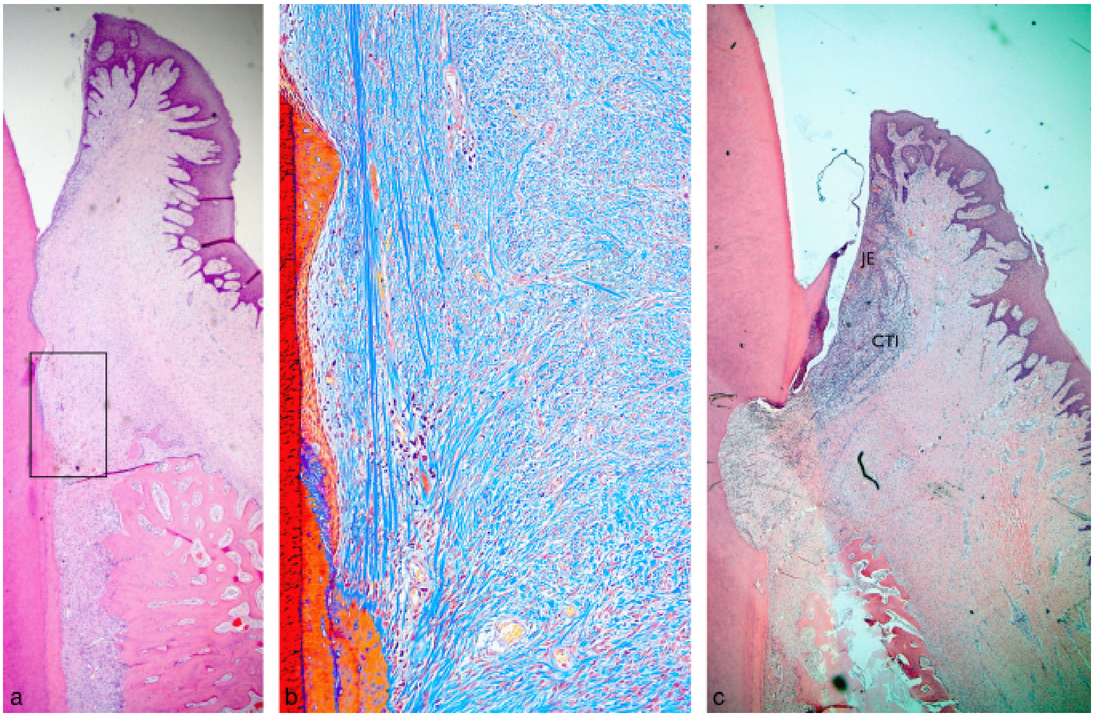

Fig. 4. (a) Cross-section of the buccal dento-gingival region representing the test group after 1 month of healing. Haematoxylin and eosin (HE) stain. Original magnification $\times$ 20. (b) Detail of (a). Fan-like distribution of the collagen bundles. Mallory's connective tissue stain. Original magnification $\times$ 100. (c) Cross-section of the buccal dento-gingival region representing the control group after 1 month of healing. Please note the connective tissue infiltrate (CTI) lateral to the junctional epithelium (JE). Haematoxylin and eosin (HE) stain. Original magnification $\times$ 20.

groups at 1 month. At 3 months of healing, however, the test group demonstrated an increased length of newly formed cementum (aN – cC) compared with the control group, although these differences were not statistically significant.

New bone formation

The differences in the distance between the apical end of the notch and the most coronal level of the bone crest are the result of the process of modelling and remodelling of the alveolar crest during post-operative healing (aN – Bc). At 1 month, the net result was positive in both treatment groups, whereas at 3 months, the test group depicted minimal bone loss while the control group showed minimal gain. The differences between groups were not statistically significant.

Gingival width

At 1 month, the width of the gingival tissues (G – width) was larger in the control group, this difference being statistically significant. At 3 months, however, the differences between groups were minimal and insignificant.

Discussion

The use of xenogeneic collagen devices has been widely investigated in different fields of dentistry, but mainly as barrier membranes for both guided bone regeneration (Hämmerle & Jung 2003) and guided tissue regeneration procedures (Sculean et al. 2008). Recently, a new collagen device has been specifically designed for soft-tissue regeneration and it has been clinically evaluated in the treatment of recession defects (Mc Guire & Scheyer 2010) and for keratinized tissue augmentation (Sanz et al. 2009, Nevins et al. 2010).

From a clinical point of view, the reduction of the recession and the increase in keratinized tissue are key outcome variables in the treatment of gingival recessions. In this experimental study, the combination of a CM and the CAF procedure significantly reduced the recession and increased the width of KT, although these outcomes were not significantly different between the test and the control groups. Nevertheless, the width of KT at the end of the study was about 1 mm greater in the control group and this value may have clinical significance. The CAF technique alone has been described as an effective procedure to attain and maintain root coverage and also demonstrated significant increases in the width of KT (Zucchelli & de Sanctis 2000, Zucchelli & de Sanctis 2005). This outcome has been justified due to the genetically determined position of the mucogingival line, which, with time, reaches its original position and consequently causes an increase of KT. The results from these clinical studies may in part justify the outcomes obtained in this investigation. Furthermore, the limitations of this animal model due to the lack of adequate measures of plaque control and post-operative care have to be taken into consideration when evaluating clinical outcomes.

Histologically, three healing periods were evaluated. At 1 week, the CM was well tolerated within the connective tissue, without eliciting pronounced inflammatory/foreign body reactions. The presence of immature fibroblasts and a high density of vascular channels within the matrix were observed. This

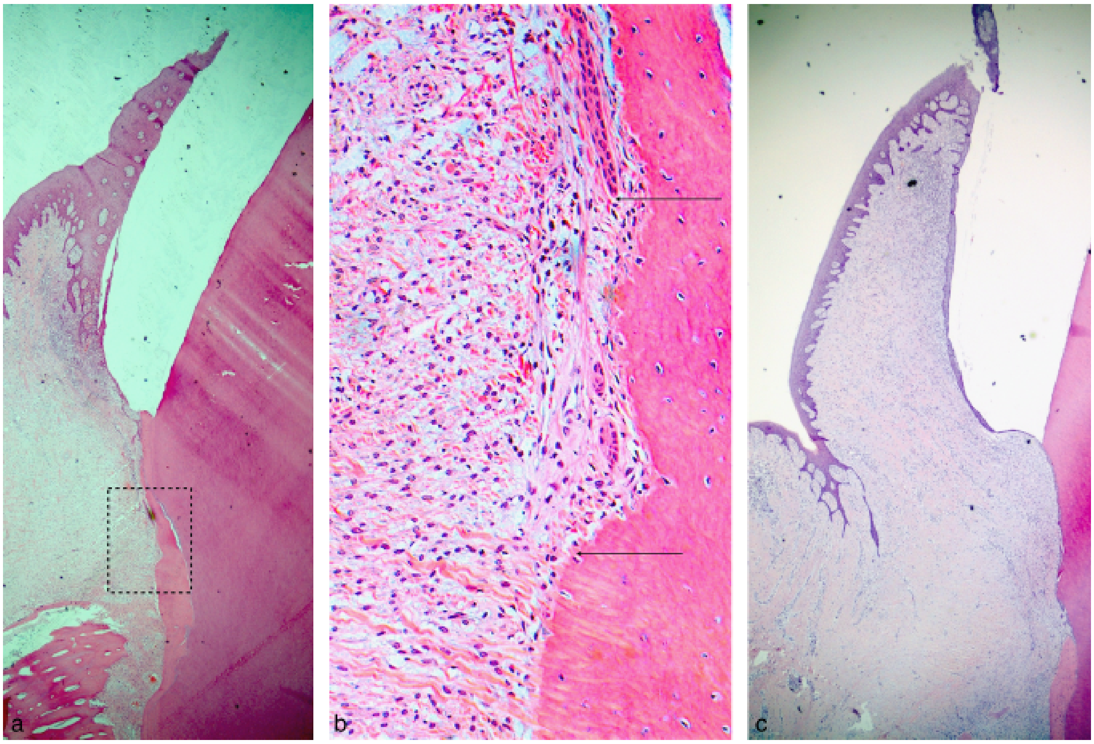

Fig. 5. (a) Cross-section of the buccal dento-gingival region representing the test group after 3 months of healing. Haematoxylin and eosin (HE) stain. Original magnification × 20. (b) Detail of (a). The zone of connective adhesion is interposed in between the most apical portion of the junctional epithelium and the inserted connective tissue fibres (arrows). Haematoxylin and eosin (HE) stain. Original magnification × 100. (c) Cross-section of the buccal dento-gingival region representing the control group after 3 months of healing. Haematoxylin and eosin (HE) stain. Original magnification × 20.

Table 2. Length of the junctional epithelium Pm–aJE [mean (SD) mm]

Healing period	Pm–aJE		cC–aJE		aN–cC		aN–Bc		G–width	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
1month	2.31 (1.11)	2.34 (0.67)	0.07 (0.10)*	0.04 (0.07)	0.10 (0.22)^	0.08 (0.22)^	0.39 (1.59)	0.04 (0.17)	0.56 (0.36)*¶	1.11 (0.21)¶
3 months	2.26 (0.23)	2.79 (0.84)	0.28 (0.32)*	0.12 (0.16)	1.08 (0.41)^	0.75 (0.25)^	– 0.20 (0.75)	0.16 (0.54)	1.30 (0.42)*	0.98(0.30)

Pm, most coronal marginal mucosa; aJE, most apical extent of the junctional epithelium. Length of the connective tissue adhesion cC–aJE [mean (SD) mm]. cC, most coronal extent of the cementum; aJE, most apical extent of the junctional epithelium. Length of the new cementum formation aN–cC [mean (SD) mm]. aN, most apical portion of the notch; cC, most coronal extent of the cementum. New bone formation aN–Bc [mean (SD) mm]. aN, most apical portion of the notch; Bc, most coronal level of the bone crest. Width of the gingiva at the mid-central portion of the junctional epithelium G–width [mean (SD) mm].

*$p < 0.005$;

^$p < 0.001$, Intergroup Diff. St.Sig;

¶$p < 0.001$.

proliferative reaction was not found in the control group. This finding is consistent with the in vitro testing of this prototype that showed the in-growth of primary human fibroblasts into the CM, which resulted in an increased expression of extracellular matrix proteins such as collagen type I and fibronectin (Mathes et al. 2010). At 1 month, the tissues adjacent to the CM presented a higher degree of maturation when compared with the control group. The CM could not be identified with the standard HE and Mallory's connective tissue stains, thus demonstrating the full integration of the xenograft into the adjacent connective tissue. After 3 months, the tissues reached complete normalcy, with no differences between the treatment groups. Recently, the CM used in this investigation and another prototype with a different source of collagen were compared for their behaviour in a non-submerged healing environment in combination with the apically repositioned flap (Jung et al. 2011). Clinical results demonstrated an increase in the width and thickness of the keratinized tissue. The qualitative histological analysis revealed complete healing of both CM, resulting in mature mucosal and submucosal tissues. In contrast, Wei et al. (2002) reported on the unsubmerged healing of ADM that resulted in a very dense connective tissue, rich in elastin fibres, with a histological appearance very different from mature gingival tissue or oral mucosa.

The histometric results demonstrated that both surgical procedures resulted in the formation of new cementum and connective tissue attachment to a previously exposed root surface. When both treatments were compared, however, a different soft tissue interface was observed. At 1 month, all histometric measurements were similar in both groups. At 3 months, however, in the control group, the junctional epithelium continued its apical proliferation and reached almost the level of the notch, whereas in the test group, the junctional epithelium remained at the same level. This resulted in a significantly shorter junctional epithelial dimension in the test group [2.26 (SD 0.23) mm] compared with the control [2.79 (SD 0.84) mm]. Furthermore, the amount of newly formed cementum was higher in the test group [1.08 (SD 0.41) mm] than in the control group [0.75 (SD 0.25) mm], although these differences were not statistically significant. A similar healing response has been reported by Casati and Sallum (2000), demonstrating a reduced apical migration of the junctional epithelium and a more pronounced new cementum formation using a bioabsorbable polylactic acid membrane in a canine model. The histological outcomes observed in the present investigation may indicate that the CM combined with the CAF is more effective in promoting new cementum formation and limiting the epithelium proliferation, than the CAF alone, thus suggesting a barrier-like behaviour.

In summary, within the limitations of this animal experiment, the tested CM used as a xenograft combined with the CAF allowed an uneventful healing, the matrix being completely incorporated into the adjacent host connective tissues, in the absence of a significant inflammatory response. The healing was characterized by the formation of new cementum and new connective tissue attachment in the apical aspect of the defect and by a junctional epithelium in its most coronal third. When compared with the CAF alone, both techniques rendered similar clinical outcomes, although the CM graft attained more tissue regeneration, with a shorter epithelium and a larger new cementum formation.

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Clinical relevance

Scientific rationale for this study: Recently, a xenogeneic CM of porcine origin has been developed as a soft tissue substitute. Still, there is no information on the type of histological healing that occurs when the CM is used in the treatment of localized Miller class-I gingival recessions with the CAF.

Principal findings: The CM combined with the CAF allowed an uneventful healing, the matrix being completely incorporated into the adjacent host connective tissues. When compared with the CAF alone, both techniques rendered similar clinical outcomes, although the CM graft attained more tissue regeneration, with a shorter epithelium and larger new cementum formation.

Practical implications: The new CM may be considered safe and effective in the treatment of gingival recessions. It may also have the potential to promote more tissue regeneration.