Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 523–531 doi: 10.1111/j.1600-051X.2009.01401.x

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Clinical and histological evaluation of an acellular dermal matrix allograft in combination with the coronally advanced flap in the treatment of miller class I recession defects: an experimental study in the mini-pig

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Núñez J, Caffesse R, Vignoletti F, Guerra F, San Roman F, Sanz M. Clinical and histological evaluation of an acellular dermal matrix allograft in combination with the coronally advanced flap in the treatment of miller class I recession defects: an experimental study in the mini-pig. J Clin Periodontol 2009; 36: 523–531. doi: 10.1111/j.1600-051X.2009.01401.x.

Abstract

Objectives: To study the wound healing of acellular dermal matrix (ADM) allografts when used together with coronally advanced flaps (CAF) in the treatment of localized gingival recessions in the mini-pig experimental model.

Material and Methods: Dehiscence defects 4 × 5 mm were surgically created in one buccal root surface in each quadrant of PI, II, or III in three mini-pigs. They were then treated with CAF and the interposition of either a connective tissue graft (CTG) or ADM. As the primary outcome, the histological interface between the ADM and the root surface was studied and was compared with CTG. As secondary outcomes, we assessed the amount and quality of the keratinized tissue and clinical outcomes in terms of root coverage and recession reduction.

Results: At 3 months, the CTG group attained a mean 76% root coverage, *versus* 62% in the ADM group. The histological interface with the root surface was similar in both groups. The apical migration of the epithelium was 1.79 ± 0.46 mm for the CTG and 1.21 ± 0.35 mm for ADM. Newly formed cementum was observed with both treatments. New bone and a newly formed periodontal ligament were shown in five specimens in the ADM group and in three in the CTG group.

Conclusion: Both materials showed similar clinical and histological outcomes.

Key words: acellular dermal matrix; gingival recession; root coverage

Accepted for publication 21 February 2009

Conflict of interest and sources of funding statement

The authors declare that they have no conflict of interests.
This study was partially sponsored by a research grant from BioHorizons®.

Gingival recession is defined as the displacement of the gingival margin apical to the cemento-enamel junction (CEJ) with oral exposure of the root surface. Even though its aetiology is still under discussion, mostly with regard to the role of traumatic tooth brushing (Rajapakse et al. 2007), it remains a highly prevalent problem (Hugoson & Norderyd 2008) with increasing treatment demands, due to its impact on aesthetics and on the likelihood of causing hypersensitivity or favoring the development of root caries.

There are multiple periodontal plastic surgical approaches documented in the literature for the treatment of gingival recession defects. Although all these techniques have shown a consistent potential for root coverage, meta-analyses from recent systematic reviews (Roccuzzo et al. 2002, Clauser et al. 2003, Oates et al. 2003) revealed a large degree of variability in clinical outcomes. The bi-laminar techniques with the interposition of a connective tissue graft (CTG as described by Langer & Langer 1985) yielded the greater percentage of root coverage (around 85%) and a larger amount of roots completely covered (around 55%). In spite of these rather predictable clinical outcomes with the use of the CTG, its healing process and histological outcome still remain controversial, because for obvious ethical reasons studies evaluating human histology after the use of these techniques are scarce. Following successful root coverage with CT grafts, healing occurs primarily by the formation of a long junctional epithelium or a connective tissue adhesion interface between the root surface and the graft (Bruno & Bowers 2000). This interface, however, has been shown to be stable over time and to resist the penetration of a periodontal probe at standard pressure. True periodontal regeneration with interposition of Sharpey's fibres has been observed only at the most apical portion of the recession defect (Harris 1999a).

In an attempt to increase the success rate of root coverage procedures, many clinicians have attempted to modify or to combine different surgical approaches, such as the coronally advanced flap (CAF) with the sub-epithelial or CTG (Wennström & Zucchelli 1996), or the use of regenerative procedures in combination with CAF, such as the interposition of a non-resorbable barrier membrane between the root surface and the flap (Pini Prato et al. 1992), a bioresorbable barrier membrane (Roccuzzo et al. 1996), enamel matrix derivatives (EMD) (Rasperini et al. 2000, Castellanos et al. 2006) or application of a platelet-rich gel (Keceli et al. 2008). Clinical studies comparing these combined surgical approaches with the standard bilaminar technique (CTG) failed to demonstrate an added benefit in terms of root coverage, although in some instances they provided increased width of keratinized tissue or tissue thickness. The CTG, in spite of its main drawback, the need to harvest an auto-graft from the palate with its inherent morbidity and the need for a second surgical procedure, still remains the standard of therapy for the treatment of localized gingival recessions.

An alternative approach in the treatment of these lesions, with the purpose of avoiding the need for harvesting an autograft from the palate, has been the use of an acellular dermal matrix (ADM) allograft. This allograft was originally intended for covering burn wounds. It is a structurally integrated basement membrane complex (BMC) and extracellular matrix in which collagen bundles and elastic fibres are the main components. Its intended mechanism of action is by acting as a three-dimensional scaffold that allows the in-growth and repopulation of fibroblasts, blood vessels and epithelium from surrounding tissues. This original graft matrix will then eventually degrade by the production of new connective tissue and will become completely replaced by host tissues (Wei et al. 2002). In clinical studies where this allograft has been compared with CTG or CAF for the treatment of gingival recessions, the use of ADM provided similar clinical outcomes in terms of root coverage (Aichelmann-Reidy et al. 2001, Tal 1999, Côrtes Ade et al. 2004, de Queiroz Côrtes et al. 2007, Andrade et al. 2008). In fact, a recent systematic review by Gapski et al. (2005) reported results from a meta-analysis from selected studies comparing ADM and CTG in the treatment of root recessions. In terms of percentage of root coverage and amount of keratinized tissue, there were no statistically significant differences between both procedures, although CTG tended to increase the width of keratinized tissue compared with ADM (0.52 mm difference; $p = 0.16$).

With regard to the healing outcomes of this allograft when used for root coverage, there are only case reports where human histological data have been provided. The ADM specimens had a similar histological outcome when compared with the CTG specimen. Both CTG and ADM grafts were, however, placed in teeth with a hopeless prognosis and therefore, this situation may not represent the best model to test the wound healing of ADM or any other graft material used in the treatment of localized gingival recession defects (Cummings et al. 2005).

The purpose of this animal experimental study is to further study and understand the predictable wound healing that occurs when ADM allografts are used together with CAF in the treatment of localized gingival recessions. The primary outcome of this histological study is to assess the interface between the ADM graft and the root surface in Miller's Class I gingival recessions (Miller 1985) surgically created in mini-pigs and to compare this outcome with that obtained when a CTG was placed under the flap in control sites. As secondary outcomes, we have assessed the amount and quality of the keratinized tissue obtained as well as the clinical outcomes in terms of root coverage and recession reduction.

Material and Methods

Animals

Three healthy 4-year-old female minipigs without periodontal disease, weighing between 86.5 and 93.5 kg, were used in this study. The protocol design and surgical procedures were approved by the Ethical Research Committee of the Hospital Central de la Defensa ''Gomez Ulla'' in Madrid.

Surgical creation of the experimental defects

Once the animals underwent the prescribed quarantine period, supra- and subgingival deposits from pre-molars PI, II, III and IV were removed with the use of ultrasonic scalers and manual curettes under general anaesthesia in order to treat existing gingival inflammation.

Dehiscence defects were then surgically created in PI, II, or III (based on the presence of a minimum width of 3 mm of keratinized gingiva) at one buccal root surface in each of the four quadrants. All surgical procedures were performed under general and local anaesthesia under sterile conditions. As pre-medication, we used Carazolol 0.2 ml/10 kg and Azaperona 0.5 ml/10 kg, which were administered intramuscularly. General anaesthesia was then achieved using Midazolam 0.5 ml/10 kg and local anaesthesia with 0.5% Articaine with epinefrine 1:100,000. The surgical design included two vertical incisions along the mesio-buccal and disto-buccal line angles of each root (5 mm apart and extending 7 mm apically) and the rise of a mucoperiosteal flap. The alveolar bone covering the

buccal root surfaces was then removed with chisels, until creating a defect between the crestal bone and the CEJ of at least 6 mm. The flaps were then apically positioned and sutured, leaving the root surfaces exposed. The dehiscence defects created were approximately 4 mm in depth and 5 mm in width, with a remaining apical band of 3 mm of keratinized gingiva (Fig. 1).

Root coverage surgery

This surgical procedure was carried out approximately 1 month after the surgical creation of the experimental defects (Fig. 2). Following the same anaesthetic protocol as described above, first, a notch was prepared at the level of the gingival margin on the buccal root surface and then the following clinical measurements were recorded: probing depth (PD), clinical attachment level (CAL), width of the keratinized gingival (KG) at the mid-buccal root surface, vertical length of the recession (VR) (measured from the gingival margin to the CEJ) and width of the recession (WR) (measured mesio-distally at the level of the CEJ). After a thorough root planning with the use of Gracey curettes, an intra-sulcular incision was made on the buccal aspect of the test and control teeth and connected to horizontal incisions in the inter-proximal area at the CEJ level. Vertical incisions were then made at the mesial and distal ends of the horizontal incisions and extended as apically as necessary for access and subsequent coronal advancement of the flap. A split-thickness flap was then elevated by means of sharp dissection as close to the periostium as possible. The distance between the osseous crest and the CEJ was measured with a periodontal probe. Randomized by the toss of a coin, the gingival recessions were treated either with a conventional autogenous CTG harvested from the palate in the control side (Fig. 3) or, in the test side, an ADM graft of porcine origin provided by Life Cell Corp. (Branchburg, NJ, USA) (Fig. 4). The CTG had an average thickness of 1 mm was trimmed to fit the defect and sutured into place using 5.0 polyglactin (Vicryl®, Ethicon, Somerville, NJ) sutures. The ADM allograft, also of an average thickness of 1 mm, was prepared following the manufacturer's guidelines and trimmed to fit the area, placing the basement membrane towards the root surface and sutured into place with 5.0 polyglactin (Vicryl®) sutures. Following the stabilization of the grafts, the reflected gingival flaps were coronally advanced to fully cover the grafts and sutured using 5.0 polyglactin (Vicryl®) sutures.

Fig. 1. The gingival recession was created surgically on the buccal aspect of the root surface having 4mm in depth, and 5 mm in width and 3mm of keratinized gingiva apically.

Fig. 2. After one month of the experimental injury the gingival recession appeared over the buccal root surface.

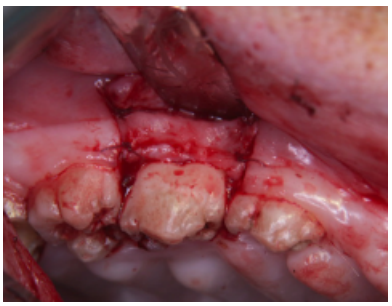

Fig. 3. A conventional Autogenous Connective tissue graft (CTG) in the control side was obtained from the palate, trimmed to fit the defect (the thickness of the graft was ± 1mm) and sutured into place using 5.0 polyglactin (Vicryl®) sutures.

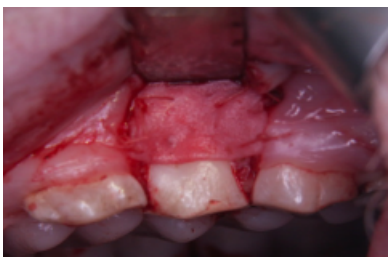

Fig. 4. An acellular dermal matrix (ADM) was placed in the test side and it was prepared following the manufacturer's guidelines and trimmed to fit the area. Each matrix specimen approximately 1mm thick, was oriented with the basement membrane adjacent to the root surface and sutured into place with 5.0 polyglactin (Vicryl®) sutures.

Post-surgical care

All mini-pigs received antibiotics post-surgically (Amoxicillin 15 g, 5 ml/50 kg) and anti-inflammatory medication (flunixin–neglumine 1 ml/kg) intramuscularly. The mini-pigs were then fed with a soft diet for 2 weeks in order to reduce potential mechanical trauma to the surgical sites. Every 21 days, the mini-pigs received an oral prophylaxis and the application of a 0.12% solution of chlorhexidine–gluconate on the tooth surfaces.

Histological processing

Three months after the root coverage surgical procedures, the animals were sacrificed with an overdose of sodium thiopental. Before sacrifice, the same clinical measurements recorded at baseline were registered. All defects in the test and control groups were dissected along with the surrounding soft and hard tissues. Block sections were fixed in 10% buffered formalin and methanol PRS for 15 days and then prepared for ground sectioning according to the methods described by Donath & Breuner (1982). In brief, the specimens were dehydrated in graded series of ethanol, embedded in hydroxy–ethyl–methacrylate and polymerized by ultraviolet light using a 450 nm wavelength.

The blocks were cut in a buccolingual plane using a cutting-grinding unit (Exakt®, Exakt Apparteau, Norderstedt b. Hamburg, Germany). Sections were stained with toluidine blue, pyromine G and H&E at intervals of 30–50 μm. From each specimen, two sections were used for histological and histometric analysis.

Histological methodology

All specimens were analysed histologically and histometrically under a light microscope (Eclipse E800, Nikon Inc., Tokyo, Japan) equipped with a compu-

terized image analysis system (NIS Elements BR, Nikon DS-Ri1). A well-trained examiner (R. C.) carried out the histological evaluation and histometric analysis blindly.

The following measurements were registered at the buccal aspect of the root in each section:

- Length of the epithelium: distance between the gingival margin and the apical end of the juctional epithelium (GM–JE).
- Length of the connective tissue adhesion: distance between the most coronal cementum and the most apical extent of the juctional epithelium (CC–JE).
- Gingival thickness at the CEJ level.
- Defect height (recession): distance between the notch and the CEJ (notch-CEJ).
- New cementum formation: length of the newly formed cementum coronal to the notch (AC–CC).
- New bone: length of the newly formed bone coronal to the notch.

Data Analysis

The means and standard deviations for each histological parameter were calculated for the experimental and control groups for each animal and the overall mean values were determined. Because of the limited sample size, differences between two groups were analysed using the Mann–Whitney test using the mini-pig as the unit of analysis ($n = 3$). Also, the Mann–Whitney test was used to compare the effect of both surgical procedures on the measured clinical parameters (clinical attachment gain, probing pocket depth, width of the recession and width of the keratinized tissue).

Results

Clinical parameters

Healing occurred uneventfully in the three mini-pigs. Four weeks after surgery, no visible adverse reactions, such as infection or suppuration, were observed (Fig. 5). At baseline, differences between the control and test groups for all tested clinical parameters were not statistically significant (Table 1). The changes in these parameters between baseline and 3 months are also depicted in Table 1. At 3 months, there were no significant differences between both treatment groups in any of the clinical parameters tested. There was a statistically significant reduction ($p < 0.05$) in the length of the recession (VR) for both groups. In the CTG group, the recession changed from 2.16 ± 0.16 mm pre-operatively to 0.5 ± 0.22 mm post-operatively, and from 2.16 ± 0.3 to 0.83 ± 0.4 mm in the ADM group. The width of the recession was also reduced significantly ($p < 0.05$) in the CTG group, changing from 4.08 ± 0.45 to 0.83 ± 0.4 mm. In the ADM group, however, changes in width were not significant (from 4.5 ± 0.42 to 2.5 ± 1.2 mm). The amount of keratinized tissue (KG) did not increase significantly in any of the groups. In terms of the main clinical outcomes tested, the CTG group attained a mean 76% root coverage, while the corresponding value in the ADM group was 62%, these differences not being statistically significant. Similar results were also obtained

Table 1. Mean (and SD) values of clinical parameters at baseline and 3 months

	CTG ($n = 3$)		ADM ($n = 3$)	
	baseline	3 months	baseline	3 months
VR	2.16 ± 0.16	0.50 ± 0.22*	2.16 ± 0.30	0.83 ± 0.40*
CAL	3.80 ± 0.16	2.00 ± 0.00*	3.60 ± 0.21	2.00 ± 0.36*
PD	1.60 ± 0.21	1.50 ± 0.22	1.50 ± 0.22	1.16 ± 0.16
KG	2.66 ± 0.42	3.83 ± 0.47	2.80 ± 0.30	3.00 ± 0.36
WR	4.08 ± 0.45	0.83 ± 0.40*	4.50 ± 0.42	2.50 ± 1.20

*Changes between baseline and 3 months are statistically significant at $p < 0.05$.
ADM, acellular dermal matrix; CAL, clinical attachment level ; CTG, connective tissue graft; KG, keratinized gingival; PD, probing depth; SD, standard deviation; VR, vertical length of the recession; WR, width of the recession.

Table 2. Clinical outcome measurements at 3 months post-surgery

3 months	CTG ($n = 3$)	ADM ($n = 3$)
Recession reduction (mm)	1.66 ± 0.21	1.33 ± 0.49
Root coverage (%)	76	62

*Differences between groups are *not* statistically significant at $p > 0.05$.
ADM, acellular dermal matrix; CTG, connective tissue graft.

Fig. 5. Three months after the root coverage surgical procedure over the first premolar the healing was uneventful.

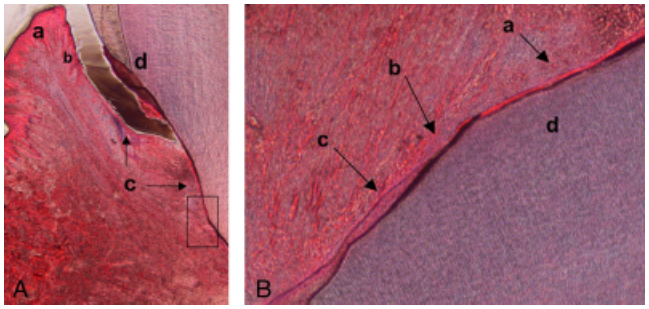

Fig. 6. A, Connective tissue specimen with parakeratinized epithelium covering the gingiva (a), Sucular epithelium (b), short junctional epithelium (c) and the CEJ (d). (original magnification × 40; H&E). B, higher magnification of the framed area in Fig 6A with junctional epithelium (a), connective tissue adhesion over root surface (b), new cementum (c) and dentin (d) (original magnification × 100; H&E).

with regard to recession reduction (1.66 ± 0.21 mm in the CTG group *versus* 1.33 ± 0.49 mm in the ADM group) (Table 2).

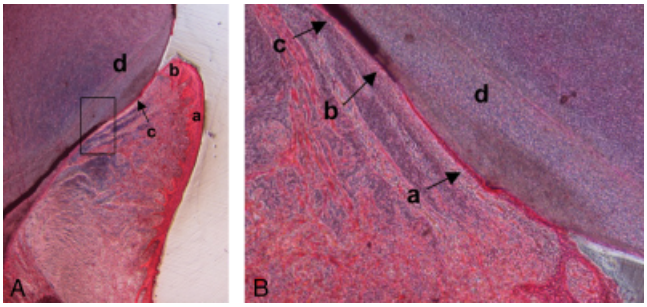

Fig. 7. A, acellular dermal matrix specimen with parakeratinized epithelium covering the gingiva (a), Sucular epithelium (b), short junctional epithelium (c) and dentin (d). (original magnification × 40; H&E). B, higher magnification of the framed area in Fig 7A with junctional epithelium (a), connective tissue adhesion over root surface (b), new cementum (c) and dentin (d) (original magnification × 100; H&E).

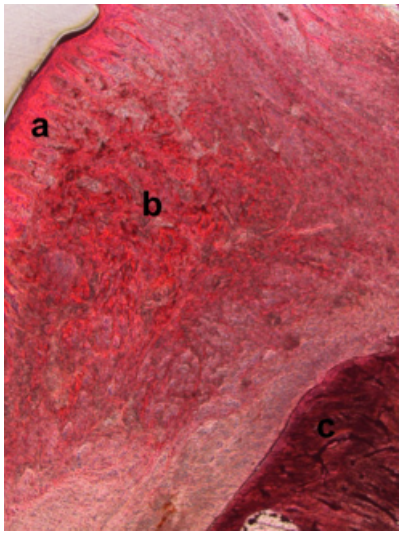

Fig. 8. Connective tissue specimen with parakeratinized epithelium (a) covering the connective tissue graft (b) and alveolar bone (c) (original magnification × 100; H&E). The differences between the connective tissue of the graft and overlying gingiva can not be ascertained.

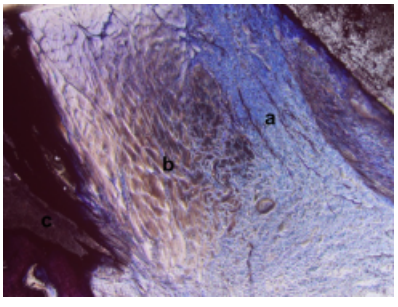

Fig. 9. Acellular dermal matrix specimen demonstrating mucosal tissue (a) overlying the ADM graft and alveolar bone (c) (original magnification × 100; TB).

Histological and histometrical findings

Histological observations were carried out in all recessions of the three animals (six specimens for the control group and six for the test group). The histological findings were similar in both groups. A para-keratinized epithelium covered the gingival margin. At the sulcus side, the apical migration of the epithelium was rather short (mean 1.79 ± 0.46 mm for the CTG and 1.21 ± 0.35 mm for ADM) (Figs. 6A, B, and 7A, B). In both groups, there was an inflammatory infiltrate limited to the connective tissues adjacent to the sulcular and junctional epithelium. In the CTG group, the interface between the connective tissue from the graft and from the gingival compartment could not be ascertained (Fig. 8). In the ADM group, conversely, remnants of the allograft were clearly identified, since at 3 months the matrix was not completely reabsorbed (Fig. 9). These remnants, however, were fully integrated with the adjacent connective tissue, being difficult to differentiate both tissues with standard haematoxylin–eosin and were not associated with any sign of inflammation (Fig. 10). In both groups, the supracrestal connective tissue fibres ran perpendicular to the root surface and inserted into the newly formed cementum (Fig. 11A and B). No signs of root resorption or ankylosis were observed in either group. Newly formed cementum on the previously denuded and contaminated dentin surface was observed with both treatments. In the ADM group, five specimens showed new bone and a newly formed periodontal ligament, coronal to the notch (Fig. 12A and B). A similar finding was seen in the CTG in only three specimens (Fig. 13A and B)

Table 3 shows the histometric analysis depicting the changes in the measured parameters after 3 months of healing. There were no significant differences between both treatment groups.

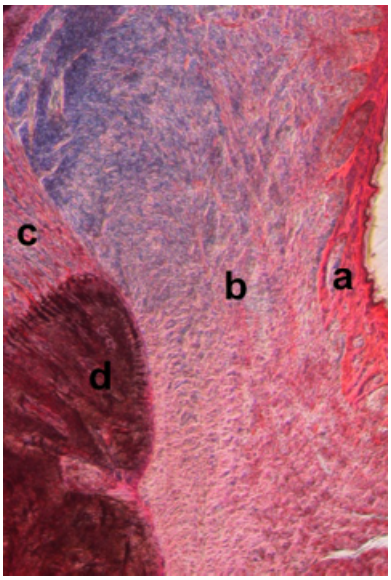

Fig. 10. Acellular dermal matrix specimen with parakeratinized epithelium (a) covering the AMD graft (b), periodontal ligament (c) and alveolar bone (d) (original magnification × 100; H&E). The grafted ADM and the overlying connective tissue are similarly dense and incorporated such that it is difficult to differentiate them.

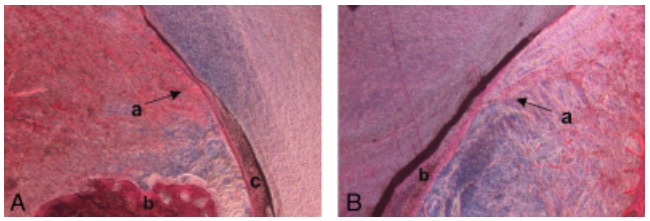

Fig. 11. A, connective tissue specimen demonstrating that the connective tissue fibers (a) in the area coronal to the osseous crest (b) inserted perpendicularly to the root surface in newly formed cementum (c). B, acellular dermal matrix also demonstrates connective tissue fibers (a) in the area coronal to the osseous crest inserted perpendicularly to the root surface in newly formed cementum (b) (original magnification × 100; H&E).

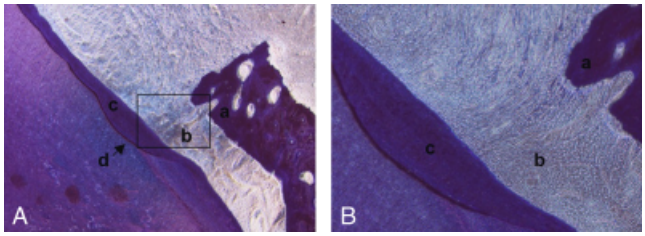

Fig. 12. A, connective tissue specimen demonstrating new bone formation (a), periodontal ligament (b), and new cellular cementum (c) within the root notch (d). (original magnification × 40; TB). B, higher magnification of the framed area in fig 11.1 alveolar bone (a) periodontal ligament and cellular cementum (c) (original magnification × 100; TB).

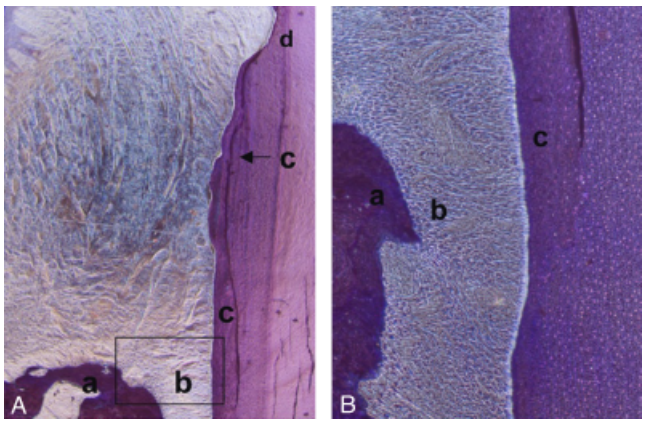

Fig. 13. A, acellular dermal matrix connective tissue specimen demonstrating new bone formation (a), periodontal ligament (b), new cellular cementum (c) and the root notch (d). (original magnification × 40; TB). B, higher magnification of the framed area in fig 11.1 alveolar bone (a) periodontal ligament and cellular cementum (c) (original magnification × 100; TB).

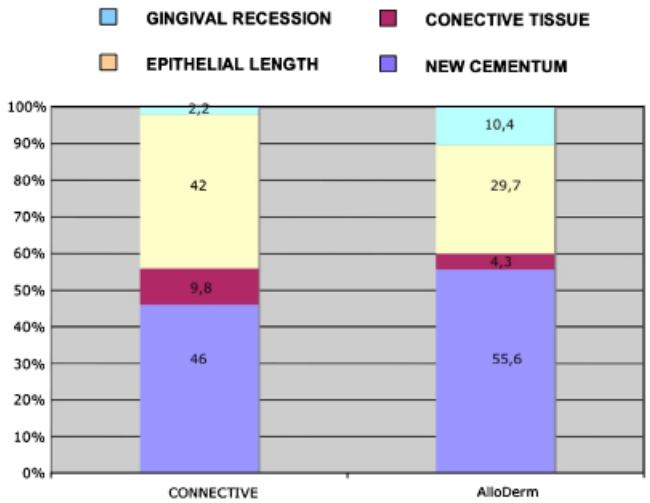

Fig. 14. Relative proportion of the tissue compartments after healing, expressed as percentage of the defect.

After 3 months of healing, the CTG group attained a mean thickness of 2.54 ± 0.93 mm, while in the ADM group, it was 1.46 ± 0.11 mm. The formation of new cementum (AC–CC) was similar in both treatment groups (2.22 ± 0.44 mm for CTG and 2.27 ± 0.42 mm for ADM). New bone formation was also similar in both groups (0.35 ± 0.49 mm for CTG and 0.61 ± 0.55 mm for ADM). The mean sulcular and junctional epithelium (GM–JE) formed (1.79 ± 0.46 mm for the CTG group and 1.21 ± 0.35 mm for the ADM group) and the amount of connective tissue adhered to the root surface (CC–JE) between the new cementum and the junctional epithelium (0.47 ± 0.44 mm for the CTG group and 0.18 ± 0.08 mm for the ADM group) were also similar in both groups. The relative proportions, expressed as percentage of the defect, of the remnant gingival recession and the lengths of the epithelium, connective tissue and new cementum, are presented in Fig. 14. The results of the healing of both the autogenous (CTG) and the allograft (ADM) were almost identical.

Table 3. Histometric results at 3 months post-surgery

mm	CTG ($n = 3$)	ADM ($n = 3$)
Notch-CEJ		
#1	2	0.99
#2	0.63	0.85
#3	1.89	0.92
Mean (SD)	1.51 ± 0.76	0.92 ± 0.07
GM–JE		
#1	2.15	1.62
#2	1.27	0.97
# 3	1.94	1.06
Mean (SD)	1.79 ± 0.46	1.21 ± 0.35
AC–CC		
#1	1.82	2.47
#2	2.13	1.78
#3	2.69	2.56
Mean (SD)	2.22 ± 0.44	2.27 ± 0.42
CEJ–osseous crest		
#1	3.71	3.47
#2	3.88	4.38
#3	2.82	2.29
Mean (SD)	3.47 ± 0.56	3.38 ± 1.04
CC–JE		
#1	0.95	0.23
#2	0.087	0.088
#3	0.37	0.23
Mean (SD)	0.47 ± 0.44	0.18 ± 0.08
Gingival thickness		
#1	1.78	1.34
#2	3.58	1.56
#3	2.26	1.49
Mean (SD)	2.54 ± 0.93	1.46 ± 0.11
New bone		
#1	− 0.21	0.52
#2	0.61	0.11
#3	0.67	1.2
Mean (SD)	0.35 ± 0.49	0.61 ± 0.55

*Differences between groups are *not* statistically significant at $p > 0.05$.
ADM, acellular dermal matrix; CEJ, cemento-enamel junction; CTG, connective tissue graft; SD, standard deviation.

Discussion

The findings of this animal experimental study indicate that regeneration, consisting of the formation of new cementum, new bone and connective tissue attachment to a previously exposed root surface can occur when placing either a sub-epithelial connective tissue autograft or an ADM allograft directly on the root surface, in combination with a CAF in experimentally created localized gingival recession lesions. Clinically, both grafting techniques attained similar results in terms of recession reduction and percent of root coverage, both being significantly different when compared with the baseline values. The biologic width attained studying both treatment groups, either with ADM or CTG was 1.39 and 2.26 mm, respectively, these results being comparable to the known biologic width dimensions around healthy teeth (Gargiulo et al. 1961).

ADM has been used as a safe soft tissue graft substitute in plastic and reconstructive surgeries. In Periodontics, it has been tested as an alternative to other periodontal plastic surgical procedures aimed to achieve root coverage in the treatment of localized gingival recessions and to increase the width of keratinized gingiva (Harris 2002). Its mechanism of action is by providing a natural scaffold made of natural collagen, which will be replaced eventually by native collagen (Luczyszyn et al. 2007). In this study, 3 months after the placement of ADM, we could identify the area corresponding to the grafted ADM, in close integration with the host connective tissue, demonstrating a similar density and with borders between the allograft and native collagen that, in some areas, were difficult to identify with standard H&E. Luczyszyn and colleagues when treating gingival recession defects in dogs with this material, have also reported this observation.

Other studies have also evaluated the histological response of ADM when used in the treatment of gingival recession (Harris 1998, Richardson & Maynard 2002, Cummings et al. 2005, Sallum et al. 2006, Luczyszyn et al. 2007). Sallum et al. (2006) reported that 41.10% of the recession defect healed with the formation of a long junctional epithelium when ADM was placed. This result is, however, different from the data obtained in this investigation where the dimension of the long juntional epithelium was 1.29 ± 0.45 mm, representing 29.7% of the defect. Conversely, these results are similar to the results reported by Casati et al. (2000) when treating similar defects with guided tissue regeneration (GTR) that showed that 28% of the defect was covered by epithelium. In fact, Tal et al. (1999) reported that the ADM graft might behave as a barrier membrane when placed between the flap and the root surface, thus preventing the downgrowth of the junctional epithelium. In this study, the dimension of the long juntional epithelium in the CTG group was 1.79 ± 0.46 mm, representing 42% of the defect. These values are comparable with the results reported previously when autogenous grafts were used in the treatment of localized gingival recession in dogs. Similar outcomes have been reported by Suaid et al. (2008), showing a healing consisting of 52.5% of epithelium, and Weng et al. (1998), with 43%.

The dimension of new cementum was 2.27 ± 0.42 mm for the ADM group and 2.22 ± 0.44 mm for the CTG group, representing 55.6% and 46% of the defect, respectively. This amount of new cementum formation obtained in both groups is similar to other histological studies that have reported 42% when using ADM (Sallum et al. 2006) and 52.8% when using CTG in combination with platelet-rich plasma (PRP) (Suaid et al. 2008). However, these authors reported that when CTG was used alone the amount of new cementum formation declined to only 26.9%, a percentage lower than what was observed in this study. Human case reports reporting histology when CTG and ADM were used as grafts in the treatment of recession defects have also reported new cementum on the apical portion of the defect, within the notch placed during the surgical procedure at the base of the defect (Bruno & Bowers 2000, Goldstein et al. 2001, Majzoub et al. 2001, Cummings et al. 2005). Other authors reporting human case reports, however, were not able to identify new cementum and in these cases the connective tissue collagen fibres did not insert to the root surface, but rather ran parallel to the surface (Harris 1999b, Richardson & Maynard 2002).

The osseous response was similar in all groups, the amount of new bone being 0.61 ± 0.55 and 0.35 ± 0.49 mm, respectively, for the ADM and CTG groups. The formation of new bone was observed in three specimens in the CTG group and in five specimens in the ADM group. This observation is in agreement with previous studies using ADM and CTG to treat periodontal recession defects (Sallum et al. 2006, Suaid et al. 2008) and studies using GTR in the treatment of recession-type defects (Gottlow et al. 1990, Weng et al. 1998, Casati et al. 2000).

An important outcome observed with this surgical procedure was not only the increase in the width of keratinized tissue but also the change in tissue thickness, moving from a thin marginal tissue with an increased risk for gingival recession to a thick gingival margin. We obtained a gingival thickness of 2.54 ± 0.93 mm for the CTG group and 1.46 ± 0.11 mm for the ADM group, thus obtaining a thicker tissue with the use of CTG grafts, in spite of the fact that both grafts had a similar thickness when placed (1 mm). The results obtained in the ADM group are, however, similar to the 1.63 ± 0.28 mm in gingival thickness reported in other studies (Sallum et al. 2006). Similarly, other authors have also reported that the sub-epithelial CTG is an effective technique for increasing the thickness of the gingival marginal tissue (Paolantonio et al. 2002).

When using an allogenic material, it is important to evaluate the inflammatory reaction from the adjacent tissues. In this investigation, there were no histological sings of chronic inflammation in any of the treatment groups, thus demonstrating that a foreign tissue reaction did not occur with the use of ADM as a graft material. A similar outcome has been reported by Novaes et al. (2007) when assessing ADM grafts in dogs. It is important to stress, however, that a porcine ADM graft was used in this study, in order to avoid the possibility of an across-species immunological response.

In summary, the results from this experimental investigation in mini-pigs indicate that when the ADM graft (AlloDerm®, BioHorizons®, Birmingham, AL) is used as an allograft for the treatment of gingival recession defects, it heals uneventfully, being incorporated by the adjacent connective tissues, without showing an inflammatory response. This healing is characterized by the formation of new cementum and new connective tissue attachment in the apical half of the defect and by a long

junctional epithelium in the most coronal third. When compared with the connective tissue auto-graft, both materials rendered similar clinical and histological outcomes, although the CTG graft attained a thicker gingival tissue. Because of the small sample size, these results should be validated with a larger sample population.

Acknowledgements

The authors wish to express their gratitude to Mrs. Claudia Brites, laboratory technician in the Department of Dental Medicine, Stomatology and Maxillofacial Surgery, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, for the processing of the histologic specimens, as well as to the Department of Experimental Surgery at Gomez Ulla Hospital for their collaboration. We also thank BioHorizons® for partially supporting this investigation.

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Clinical Relevance

Scientific rationale for the study: AMD allograft (AlloDerm®) has been evaluated clinically and has demonstrated similar clinical outcomes when compared with the connective tissue auto-graft (CTG) in the treatment of localized gingival recession. There is, however, limited information available on how this allograft heals when applied over root surfaces under a CAF. The objective of this preclinical experimental investigation was therefore to study the interface between the AMD graft and the root surface and to evaluate whether the ADM graft would have similar histological outcomes when compared with the autograft connective tissue.

Principal findings: The clinical and histological findings were similar in both groups (CTG and Alloderm®); both grafts developed a short epithelial interface at the most coronal area, the rest being composed by newly formed cementum and connective tissue adhesion. There were no signs of root resorption or ankylosis.

Practical implications: Because AlloDerm® demonstrated similar histological outcomes when compared with the CTG, it may be a good alternative in the treatment of localized gingival recessions because it avoids the morbidity associated with the donor site.