Comparison of Extracellular Matrix Membrane and Connective Tissue Graft for Root Coverage in Class I/ II Gingival Recession Defects: A Split Mouth Study

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Abstract

Objective: The aim of this study was to assess the feasibility of extracellular matrix membrane (DynaMatrix®) in obtaining root coverage and compare it to the connective tissue graft for the treatment of Miller's Class I or Class II recession defects.

Methods: Ten patients with a mean age of 31.2 years with bilateral Miller's Class I or Class II recession defects in the upper premolars were recruited. Each patient contributed two defects that were randomly treated by coronally advanced flap with connective tissue graft (CAF+CTG) and by coronally advanced flap underlaid with extracellular membrane (DynaMatrix®; CAF+DM). All the clinical parameters were recorded at baseline, three months and six months after surgery and data were statistically analyzed.

Results: The results of this study demonstrated that both the procedures were effective and predictable in root coverage procedures. However, no statistically significant differences in gingival recession reduction were noted between extra cellular membrane and gold standard connective tissue graft.

Conclusion: Within the limits of this clinical study, the use of extracellular membrane (DynaMatrix®) may represent an acceptable alternative to the connective tissue graft for treating gingival recession.

Key words: Recession, root coverage, Dynamatrix®, connective tissue graft, coronally advanced flap

Introduction

In the current practice of periodontology, clinicians are faced with the challenge of not only addressing biological and functional problems present in the periodontium, but also providing therapy that results in acceptable aesthetics. One of the most common aesthetic concerns associated with the periodontal tissues is gingival recession (Oates *et al*., 2003).

Coronally advanced flap (CAF) is the first choice of surgical technique for root coverage when there is adequate keratinized tissue apical to the recession defect. Good root coverage results, good colour blending of the treated area with respect to adjacent soft tissues, and recuperation of the original morphology of the soft tissue margin can be predictably accomplished using this surgical approach (Allen and Miller, 1989; Cortellini *et al*., 2009). However, as a stand-alone procedure it may not achieve true periodontal regeneration (Zuchelli and Sanctis, 2000) and the long-term stability of root coverage is questionable (Pini Prato *et al*., 2011).

Hence, clinical researchers are trying to achieve stable and predictable root coverage by adding a connective tissue graft (CTG) to the coronally advanced flap. This approach is considered to be the gold standard for the treatment of gingival recessions and is associated with a greater probability of obtaining complete root coverage (CRC) with a high

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predictability and without significant post-surgical complications when compared to other techniques (barrier membrane - membrane exposure may occur during healing; enamel matrix derivate - lack of periodontal regenerative cells; acellular dermal matrix - avascular; platelet-rich plasma - lack of strength; Pini Prato *et al*., 2011; Cairo *et al*., 2008; Bouchard *et al*., 2001; Griffin *et al*., 2006; Harris *et al*., 2005; Edel, 1974).

However, this auto-transplantation procedure requires harvesting the graft from a donor area, which results in an additional wound site with post-surgical bleeding and patient discomfort as common sequelae (Harris, 1997; Reiser *et al*., 1996). Occasionally, anatomic limitations in the palate make it difficult, if not impossible, to harvest the graft (Reiser *et al*., 1996).

To overcome this problem, principles of guided tissue regeneration have been applied for the treatment of soft tissue recession with the aim not only for root coverage but also to accomplish true periodontal regeneration (Travassos *et al*., 2015). In this regard, allogenic soft tissue graft material such as AlloDerm® was introduced and investigated (Novaes and de Barros, 2008). Even though good results were achieved with this membrane in terms of root coverage, it is presented with certain clinical limitations such as shrinkage of the graft, an inflammatory response that resembled a foreign body reaction, and an insignificant enhancement in the quality and quantity of the keratinized gingiva (Nevins *et al*., 2010).

In an attempt to surmount these clinical limitations, an extracellular matrix membrane, DynaMatrix® (DM; Citagenix, Laval, Quebec, Canada)) was introduced. It is derived from porcine small intestinal submucosa and consists of predominantly (90%) of collagens types I and III, similar to the dermal component of skin, and also small amounts of collagen types IV, V and VI (Lindberg and Badylak, 2001). The rationale for the use of DynaMatrix® in root coverage procedures is to stimulate regeneration of periodontal attachment on denuded roots, as it has been shown that it stimulates cell differentiation and proliferation along with basement membrane formation, and supports cellular adherence and angiogenesis. These unique properties induce keratinization over the membrane, which is similar to autogenous graft healing (Lindberg and Badylak, 2001; Nihsen *et al*., 2008). Its usage in the field of periodontics was first investigated (Nevins *et al*., 2010) as a way to increase the width of attached keratinized tissue; the results of the study suggested that it has the potential to augment the gingiva. Further, the clinical efficacy of this membrane along with demineralized freeze-dried bone graft (Dynablast) for ridge preservation procedures has been successfully documented (Nevins *et al*., 2011). We speculate that DynaMatrix® has the potential to be used for root coverage procedures. To date there are no studies evaluating the effectiveness of DynaMatrix® for root coverage procedures.

Hence, we aimed to assess whether DynaMatrix® has the potential to obtain root coverage, and to evaluate its clinical efficiency when compared to a connective tissue graft, considered the gold standard for the treatment of Miller's Class I and II recession defects.

Materials and methods

Patient and site selection

A double-blinded, split-mouth case series was designed to compare the clinical performance of the CAF with DynaMatrix® and CAF with CTG for the treatment of gingival recessions. The study was performed at the Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore.

Twelve patients were recruited; however, two were lost to follow-up. Data from 10 patients (5 males), age range 18 to 50 years (mean 31.2 ± standard deviation of 1.7 years), requiring root coverage therapy for upper first premolars for esthetic or hypersensitivity reasons were analyzed. The inclusion criteria were bilateral Miller's Class I or II recessions of ≥ 2 mm (Chambrone and Tatakis, 2015) in maxillary canines and premolars caused by traumatic tooth brushing, with no significant differences in recession, relative attachment level (RAL), keratinized tissue (KT) and gingival biotype (> 0.8 mm), probing depth of < 3 mm with no bleeding on probing, a full mouth plaque and full mouth bleeding score of < 20%. Teeth associated with recession were vital without any presence of caries, restorations, grooves or irregularities.

Exclusion criteria were systemically and periodontally compromised participants, a known allergy to any of the materials used in the study, requirement for antibiotic prophylaxis, failure to maintain an oral hygiene level ≥ 80% plaque-free surfaces, presence of cervical abrasion ≤ 1 mm at the cementoenamel junction (CEJ), pregnancy or lactation, use of tobacco products, alcohol abuse, long-term steroid therapy, failure to complete the informed consent, history of periodontal or mucogingival surgery in the experimental site in the last five years.

All eligible participants who volunteered were informed of the nature, potential risks and benefits of their participation in the study, and a written signed informed consent in accordance with the Helsinki Declaration of 1975 as revised in 2000 was obtained from them. Ethical clearance for the study was obtained from the ethical committee of Krishnadevaraya College of Dental Sciences and Hospital, affiliated with the Rajiv Gandhi University of Health Sciences.

Clinical measurements

At baseline, three-month and six-month visits, an examiner blinded to the surgical protocol recorded all the clinical outcome variables using a customized acrylic stent, which was prepared from the study model of the patients. Vertical grooves were made on the stent to guide the angulation and position of probe in the same plane every time it was inserted for recording the measurements.

The following data were collected for sites examined with the use of a University of North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA) and were rounded to the nearest 0.5 mm (one point per tooth on the mid-buccal aspect): recession height (RH) - distance from the lower border of the stent to the free gingival margin; recession width (RW) - measured at the CEJ (distance between the mesial gingival margin to distal gingival margin); probing pocket depth (PPD) - distance from the gingival margin to the base of the gingival sulcus; relative attachment level (RAL) - measured as PPD and RH; keratinized tissue width (KTW) - distance between the free gingival margin to the mucogingival junction (one point per tooth on the mid-buccal aspect); plaque index (PI) and gingival index (GI; Löe, 1967) were recorded. Standardized radiographs (using the paralleling technique with positioning device, radiographic grid and silicone-based impression material for the mechanical retention of the bite plane) were taken to evaluate interproximal alveolar bone levels to aid in the gingival recession classification of teeth exhibiting recession defects. Pre-operative and follow-up photographs were taken at each visit.

Pre-surgical phase

As part of the screening phase for inclusion, a detailed case history of each patient was recorded. Ten patients who were selected to participate in the study received a session of oral prophylaxis including scaling and root planing and instructions in proper oral hygiene measures, including correction of traumatic tooth brushing technique by the patient.

At the time of the surgery, in each patient, one of the two teeth with a gingival recession area was randomly assigned, by a coin toss, to the CAF + DM procedure and the contra-lateral tooth to the CAF + CTG procedure. The treatment allocation was concealed from the therapist by opaque envelopes that were opened upon completion of the common part of the surgical treatment (flap elevation and root planing). The clinical examiners remained blinded to the treatment assignment until after completion of the statistical analysis.

Surgical procedure

Patients were instructed to rinse with 10 mL of 0.2% chlorhexidine gluconate (Rexidine, Indoco Remedies Ltd. (Warren) India) solution. The extra-oral surfaces of the patient were swabbed with 5% povidone iodine (Betadine, Purdue Pharma, Stamford, CT) solution. The operative site was anesthetized with 2% lignocaine hydrochloride with adrenaline (1:200000; Lignox 2% A, Indoco Remedies LTD, Mumbai, India) using the block and infiltration technique. Systemic antibiotics (amoxicillin 1 g - Cipmox, Parthweb Solutions Pvt Ltd, Maharshtra, India) were given 1 hour before surgery. All surgical procedures were performed by one operator (Yogini).

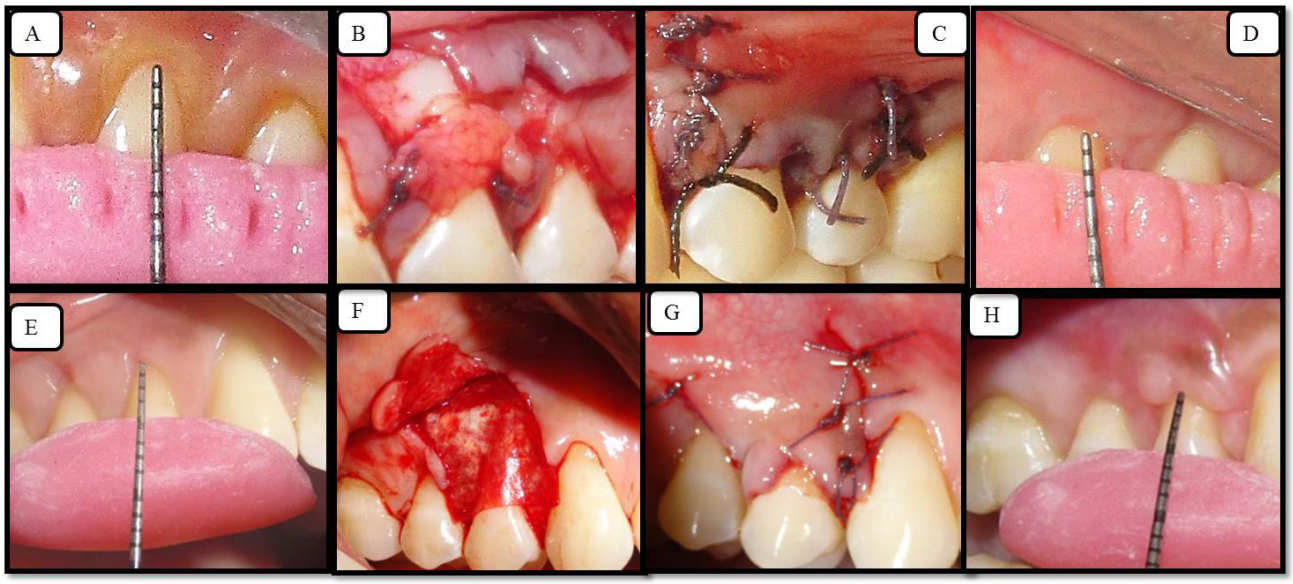

Figure 1. A) Baseline clinical photograph of the control site. The premolar (24) was to be treated with CAF + CTG; B) Flap elevated and connective tissue graft secured with sling sutures; C) Control site treated with CAF + CTG. Flap advanced coronally and stabilized using sling sutures; D) Control site treated with CAF + CTG six months after surgery; E) Baseline clinical photograph of the test site. The premolar (14) was to be treated with CAF + DM; F) Flap elevated and DynaMatrix® membrane placed; G) Test site treated with CAF + DM. Flap advanced coronally and stabilized using sling sutures; H) Test site treated with CAF + DM six months after surgery. (CAF, coronally advanced flap; CTG, connective tissue graft, DM, DynaMatrix® extracellular membrane)

Coronally advanced flap + connective tissue graft (Figure 1 A-D)

After local anesthesia, an intrasulcular incision was made at the buccal aspect of the involved tooth. Two horizontal incisions were made at right angles to the adjacent interdental papillae, at the level of the CEJ, without interfering with the gingival margin of the neighboring teeth. Two oblique vertical incisions were extended beyond the mucogingival junction (MGJ) and a trapezoidal mucoperiosteal flap was raised up to the MGJ. A split thickness flap was extended apically, releasing the tension and favoring the coronal positioning of the flap. The epithelium on the adjacent papillae was stripped away. The root surface was curetted and washed with saline solution (Cortes *et al.*, 2004; Pini Prato *et al.*, 2000).

A connective tissue graft was harvested from the palate (trap door technique; Langer and Langer, 1985; Nelson, 1987) and the graft was trimmed and shaped to fit the recipient site and was placed over the denuded root surface. The graft was stabilized using sling sutures at or slightly above the level of the CEJ using 5-0 Vicryl resorbable sutures (Ethicon, Somerville, NJ, USA) to prevent wicking. The recipient flap was coronally advanced with sling suturing to completely cover the CTG with very little tension on the flap, and interrupted sutures were placed on the releasing incisions in an apico-coronal direction, using 5-0 Vicryl resorbable sutures. The treated area was protected using a non-eugenol dressing (Coe Pack, GC, America Inc., IL, USA).

Coronally advanced flap + DM site (Figure 1 E-H)

Preparation of the membrane recipient site was similar to the CAF + CTG site. In the CAF + DM site, the DynaMatrix® membrane was adapted after being aseptically rehydrated in sterile saline according to the manufacturer's instructions. A template was prepared and the membrane was trimmed to the shape and the size of the template designed to cover the root surface and the adjacent surrounding bone. The membrane was placed over the defect area to completely cover the recession up to the CEJ and laterally by 2 mm on both sides of the recession defect. After placement of the extracellular membrane, the flap was coronally positioned and sutured in a manner similar to the CAF + CTG site and the treated area was protected using a non-eugenol dressing.

Post-surgical protocol

All patients were prescribed systemic antibiotics amoxicillin 500 mg (thrice daily) for 7 days (Cipmox, Parthweb Solutions Pvt Ltd) post-operatively (Zanicotti *et al.*, 2009). The patients applied ice packs for 15 minutes per hour for the 4 first hours (Borghetti *et al.*, 1999). Patients were instructed to refrain from brushing and flossing around the surgical sites the first 30 days after surgery and to consume only soft foods during the first week after surgery. Patients were also instructed to avoid any mechanical trauma to the treated sites. For 4 weeks, patients used a 0.12% chlorhexidine solution rinse for 1 minute twice daily (Zanicotti *et al.*, 2009).

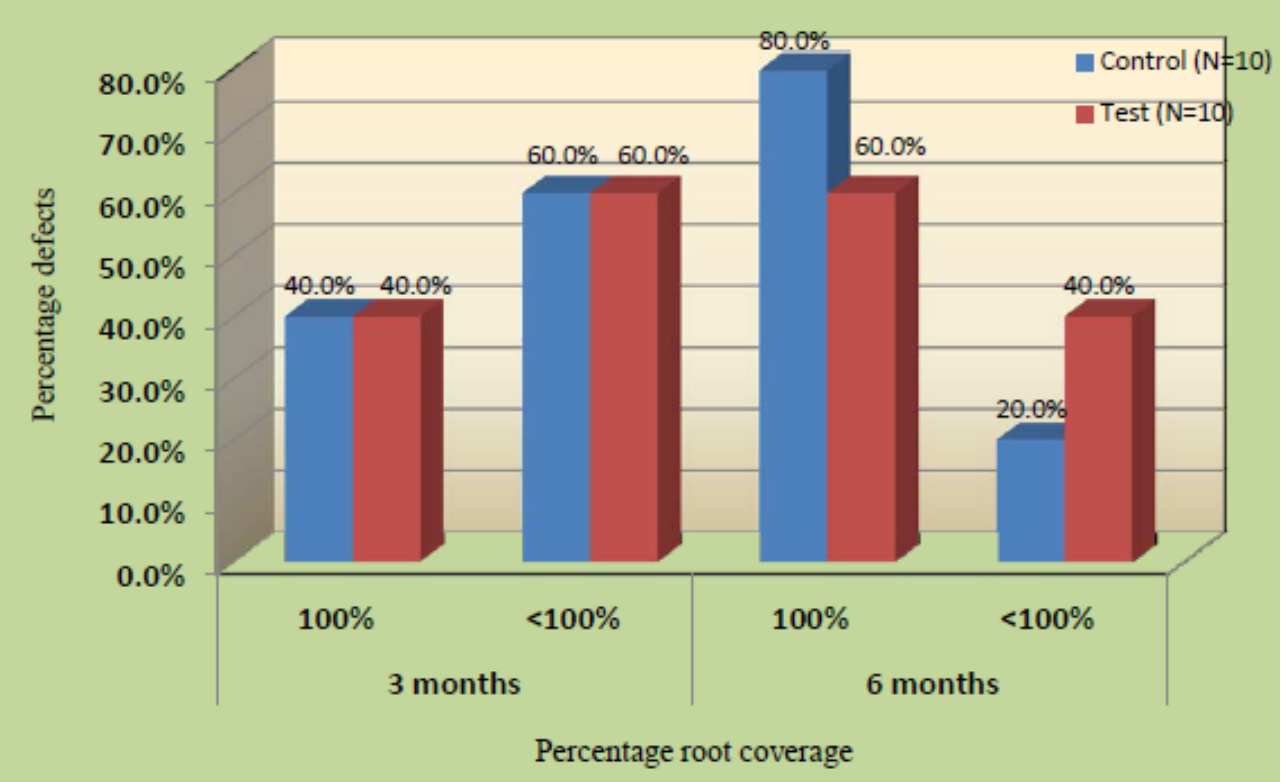

Figure 2. Comparison of percentage defects with 100% root coverage between the control CAF + CTG and test groups.

Fourteen days after surgery, the dressing was removed and the surgical sites were irrigated with normal saline. An enquiry regarding post-surgical problems was made and areas checked for any membrane exposure, in which case the periodontal dressing was replaced for another 7 days. One month after surgery, the patients were instructed to resume careful mechanical tooth cleaning of the treated areas using a soft toothbrush and a roll technique. All patients were recalled for a professional prophylaxis and plaque control once a week during the first month, bimonthly until the third month, and once a month until the final examination at 6 months.

The Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to test the normality of clinical parameters (recession width, recession height and keratinized tissue width) between baseline and 6 months. The *p* value of the Shapiro-Wilk test was below 0.05, hence the data significantly deviated from a normal distribution. The Wilcoxon signed rank test was applied to determine the difference in clinical parameters between the CAF + DM and CAF + CTG sites at 3 and 6 months post-operatively. The chi-square test was applied to analyze the gingival index and plaque index between test and control sites at baseline, 3 months and 6 months. The Mann Whitney U test was used to compare the percentage of root coverage between the control and test sites at 3 months and 6 months. Spearman's correlation coefficient was used to determine the association between baseline recession height and percentage change in recession height at 6 months (*Tables 1 - 3*).

Results

In the present pilot study, a split mouth protocol was used to balance inter-individual variations. In each patient, two surgical procedures were performed by the same operator at the same appointment. Furthermore, the inter-examiner variability was eliminated by having one masked calibrated examiner performing all measurements.

At baseline no significant differences in recession were found between CAF + DM and CAF + CTG sites ($p > 0.05$). Clinical observation at 6 months follow-up showed that both sites presented with complete healing, along with excellent color and texture blend. The extracellular membrane-treated sites blended well with the surrounding tissue and appeared esthetically pleasing. Statistically significant reductions in recession height and recession width were noted in both the CAF + CTG sites and CAF + DM sites at 3 months and 6 months post-treatment. However, when CAF + CTG and CAF + DM sites were compared there was no statistically significant difference ($p < 0.05$; *Tables 1 - 3*).

The mean increase in keratinized tissue width from

Table 1. The Wilcoxon signed rank test was used to determine the difference in clinical parameters between the test and control groups at three months and six months post-operatively.

Clinical Parameter (mm)		Control (CAF + CTG)		Test (CAF + DM)		Control/Test (Difference)	
		BL-3M	BL-6M	BL-3M	BL-6M	BL-3M	BL-6M
Recession width	Mean	-0.7	-0.7	-0.6	-0.7	-0.1	0
	(SD)	(0.483)	(0.483)	(-0.516)	(-0.483)		
	p value	0.008*	0.008*	0.014*	0.008*	0.6191	1
Recession height	Mean	-0.8	-1.3	-0.8	-1.4	0	0.1
	(SD)	(0.632)	(0.675)	(0.919)	(0.966)		
	p value	0.011*	0.003*	0.033*	0.01*	1	0.9173
Keratinized tissue width	Mean	0.8	1.4	0.6	1.1	0.2	0.3
	(SD)	(0.422)	(0.699)	(0.843)	(0.876)		
	p value	0.1326	0.0099*	0.2571	0.0404*	0.7882	0.6871
Relative attachment level	Mean	0.9	1.00	0.7	1.4	0.2	-0.4
	(SD)	(0.594)	(0.182)	(0.16)	(0.22)		
	p value	0.008*	0.007*	0.033*	0.033*	0.7983	0.5386
Probing pocket depth	Mean	0.8	0.1	0.1	0.00	0.7	0.1
	(SD)	(0.42)	(0.31)	(0.31)	(0.00)		
	p value	0.005*	0.317	1.00	1	0.3986	0.9127

BL, baseline; CAF, coronally advanced flap; CTG, connective tissue graft, DM, DynaMatrix® extracellular membrane; *$p < 0.05$. Negative values of recession width and/or height indicate a total gain when compared to baseline.

Table 2. The Mann Whitney U test was used to compare the mean values of root coverage between the control (CAF+CTG) and test (CAF+DM) groups at three months and six months.

Group	Post -operative follow-up	n	Mean	SD	Median	Min.	Max.	Mann Whitney U Test	*p* value
Control	3 months	10	65	33.7474	50	0	100	49.50	0.968
Test		10	68.33	28.8228	58.35	33.3	100		NS
Control	6 months	10	90	21.082	100	50	100	41.00	0.400
Test		10	78.3	33.38	100	0	100		NS

CAF, coronally advanced flap; CTG, connective tissue graft, DM, DynaMatrix® extracellular membrane; NS, not significant

Table 3. The chi square test was used to compare the percentage of defects with 100% root coverage between the control (CAF+CTG) and test (CAF+DM) groups at three months and six months.

Group	% Root coverage at 3 months		χ2 value	*p* value	%Root Coverage at 6M		χ2 value	*p* value
	100%	**<100%**			**100%**	**<100%**		
Control	4 40.0%	6 60.0%	0	1 NS	8 80.0%	2 20.0%	0952	0.329 NS
Test	4 40.0%	6 60.0%			6 60.0%	4 40.0%		
Total	8 40.0%	12 60.0%			14 70.0%	6 30.0%		

CAF, coronally advanced flap; CTG, connective tissue graft, DM, DynaMatrix® extracellular membrane; NS, not significant

baseline to 6 months for control and test sites was 1.40 ± 0.69 mm and 1.1 ± 0.87 mm, respectively. These values were statistically significant; however, the difference between the control and test sites was statistically insignificant.

Intra-group comparison showed that there was statistically significant reduction in relative attachment level both at 3 months and 6 months post-operatively. The mean reduction in relative attachment level from baseline to 3 months and 6 months between the control and test sites was 0.2 mm and 0.4 mm, respectively, which was statistically insignificant. There was a small increase in the probing depth that could not be considered clinically relevant because all cases showed a healthy sulcus with no bleeding on probing after 6 months. Regarding the other clinical parameters such as gingival index and plaque index, there were no statistically significant differences.

At 6 months follow-up, the control sites showed 90% percentage root coverage, and 78.3% was achieved in the test sites. The difference in the percentage root coverage between the test and control sites was not statistically significant.

Discussion

The rationale for the use of extracellular matrix membrane in root coverage procedures is to stimulate regeneration of periodontal attachment on denuded roots, as it has been shown that it stimulates cell differentiation and proliferation along with basement membrane formation, and supports cellular adherence and angiogenesis; these unique properties induce keratinization over the membrane that is similar to autogenous graft healing (Lindberg and Badylak, 2001; Nihsen *et al*., 2008). In dentistry, it has been successfully used for increasing the width of attached keratinized tissue and root coverage (Saroff, 2011).

However, to the best of our knowledge, to date there have been no clinical studies conducted to test the feasibility of using extracellular membrane in root coverage procedures compared to the gold standard connective tissue grafts. Hence, we carried out a case series that is the first of its kind to evaluate the efficiency of extracellular membrane versus connective tissue graft in a coronally advanced flap procedure for the treatment of recession.

The results of this study demonstrated that both procedures were effective and predictable in obtaining root coverage; however, there was no clinical or statistically significant difference between the connective tissue graft and extracellular membrane in recession reduction at the three-month and six-month re-evaluation visit.

The mean percentage root coverage achieved in the CAF + CTG group was 90%, and in the CAF + DM group it was 78.3%, with no significant difference between the groups. Since there are no previous studies on the use of extracellular membrane in root coverage procedures, the results obtained in the CAF + DM group were compared to other studies where different bioabsorbable membranes using the principle of GTR are used for root coverage. Genon *et al.* (2001) used a bioabsorbable collagen membrane (Geistlich Bio-Gide® Perio, Geistlich Biomaterials, Geistilich Pharma North America Inc.) in his study and found that percentage of root coverage was 74.59%, and for the acellular dermal matrix, it ranged from 65.9% (Aichelmann-Reidy *et al.*, 2008) to 91.7% (Harris, 2002). The percentage root coverage obtained in our study is on par with these other studies.

The percentage of defects with complete root coverage was 80% for the CAF + CTG group and 60% in the CAF + DM group. Studies (Roccuzzo *et al.*, 2002; Tatakis and Trombelli, 2000; Baldi *et al.*, 2009) have shown that the percentage of defects with complete root coverage is 83.3% when CTG is used in conjunction with CAF, comparable to the extracellular membrane in root coverage procedures. Borghetti *et al.* (1999), Trombelli *et al.* (2000), and Nevins *et al.* (2010) hypothesized that the epithelium that populated the extracellular membrane migrated from the denuded epithelium and induced secondary epithelialization by "creeping over" the wound bed. This is probably due to the unique scaffold, which allowed repopulation and migration of fibroblasts, blood vessels, and epithelium from the surrounding tissues.

To date, instances of early membrane exposure have demonstrated impaired regenerative outcomes. Soft tissue dehiscence resulting in spontaneous membrane exposures have been reported to range from 28% to 40%. (Nevins *et al.*, 2011) Furthermore, most bioabsorbable barrier membranes are not intended to be left exposed during the healing period. However, in our study it was seen that in one of the cases the membrane was left exposed, yet there were no adverse effects reported. At 2 weeks, early epithelialization had taken place and the tissues were devoid of any infection or inflammation during the follow-up period. Reports of uneventful healing following membrane exposure seen in this study were also reported by other studies (Nevins *et al.*, 2010; Nevins *et al.*, 2011).

However, the results have to be interpreted cautiously as there were certain limitations. Firstly, the sample size was small (n = 10). Secondly, since it was a short-term clinical trial of six months, the long-term stability of root coverage is not known. Thirdly, because the marginal tissue thickness is a critical determinant of further recession and influences the outcome of root coverage, gingival thickness, which was not evaluated in this study, should be taken into consideration. Fourthly, improving patient outcome is an important issue in clinical practice, yet the patient-related factors (pain, time and esthetics) were not evaluated in this study. Fifthly, the type of attachment gained cannot be determined without histology, as obtaining this information would require the removal of successfully treated teeth. Hence, histological examination was not carried out in this study.

In our opinion, a future multicenter, randomized control trial with a larger sample size and including histological evaluation and prolonged follow-up, along with the assessment of patient-related factors, are required to confirm the results observed in this study. Further, comparative studies have to be carried out to know the efficacy of this membrane over other membranes such as acellular dermal matrix. In addition, the feasibility of the versatile application of extracellular membrane in the treatment of angular bony defects, furcation involvement, sinus augmentation procedures and papilla reconstruction should be considered.

Individually, the surgical techniques (CAF + CTG and CAF + DM) were successful in recession reduction. However, when the root coverage techniques were compared there was no clinical or statistically significant difference in recession reduction. As use of extracellular membrane has many potential benefits, such as an unrestricted supply, avoidance of donor site morbidity, enhancement of patient acceptance (pain, time, and esthetics) and reduction of surgical challenges for the clinician, it could be considered as a viable substitute for connective tissue grafts in the treatment of recession defects.

Conclusion

Within the limits of this study, it can be concluded that there is no clinical or statistically significant difference in recession reduction between the use of extracellular matrix membrane (DynaMatrix®) and connective tissue grafts for root coverage in Millers Class I and Class II recession defects.

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