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Coverage of Miller class I and II gingival recessions treated with subepithelial connective tissue graft, acellular dermal matrix, and enamel matrix proteins. Pilot study

Recobrimento de recessões gengival classe I e II de Miller com enxerto de tecido conjuntivo subepitelial, matriz dérmica acelular, e proteína derivada da matriz do esmalte. Estudo piloto

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Resumo

Objetivo: O objetivo desse estudo foi de comparar os achados clínicos obtidos no tratamento de recessões gengivais utilizando o enxerto de tecido conjuntivo subepitelial (SECT), a matriz dérmica acelular (ADM) e as proteínas derivadas da matriz do esmalte (EMP). **Material e método:** Doze pacientes que apresentavam recessões gengival classe I e II de Miller nos caninos e pré-molares superiores foram randomicamente alocados para receber o SECT, ADM ou EMP. Análises clínicas foram executadas antes e 3 meses após os procedimentos cirúrgicos. Foram avaliados os seguintes parâmetros: porcentagem de recobrimento radicular, altura e comprimento da recessão gengival, profundidade de bolsa a sondagem, nível clínico de inserção, altura e espessura da gengiva queratinizada. O teste de Kruskal-Wallis complementado pelo teste de Dunn foram usados para avaliar as diferenças entre os grupos em cada período enquanto que o teste de Wilcoxon foi utilizado para avaliar as diferenças dentro de cada grupo variando-se o período de avaliação. Os teste foram aplicados com nível de confiança de 95%. **Resultado:** Os grupos SECT e ADM apresentaram maior porcentagem de recobrimento radicular e maior redução da altura e comprimento das recessões do que o grupo EMP (p<0,05). **Conclusão:** O SECT e a ADM são mais efetivos no tratamento de recessões gengivais que o EMP.

Descritores: Cirurgia oral; retração gengival; técnicas de retração gengival.

Abstract

Objective: The aim of this study was to compare clinical findings obtained in the treatment of gingival recessions using subepithelial connective tissue graft (SECT), acellular dermal matrix (ADM), and enamel matrix proteins (EMP). **Material and method:** Twelve patients with Miller class I and II recession in the canines or upper premolars were randomly divided into groups to receive treatments using SECT, ADM, or EMP. Clinical measurements were performed before and three months after surgical procedures. The data evaluated were as follows: percentage of root coverage, height and width of gingival recession, probe depth, clinical attachment level, and height and thickness of keratinized gingiva. The Kruskal-Wallis test complemented by Dunn's test was used to perform the between-group, analysis and the Wilcoxon test was used to perform the within-groups analysis. The tests were applied at the 95% confidence level. **Result:** The SECT and ADM groups had a higher percentage of root coverage and greater reduction in the height and width of gingival recessions compared to the EMP group (p<0.05). **Conclusion:** The SECT and ADM are more effective in treating gingival recessions than EMP.

Descriptors: Oral surgery; gingival recession; gingival retraction techniques.

INTRODUCTION

Recession of the marginal gingiva tissue is one of the most common periodontal diseases and is defined as atypical migration of the gingival margin with respect to the cementoenamel junction¹. Currently, gingival recession occurs frequently in young people and adults and is caused by various etiological factors such as inadequate brushing, plaque accumulation, occlusal problems, orthodontics, and tooth loss, among others^{1,2}. This displacement causes several problems, of which the main ones are aesthetics, especially when the anterior teeth are affected; dentin hypersensitivity, which is significant due to increasing functional deficit; difficulty in carrying out proper oral hygiene; and in extreme cases, tooth loss. Therefore, it must be treated³.

Over the years, numerous techniques have been described in the research literature on root coverage⁴⁻⁶. One of the most used surgical techniques is subepithelial connective tissue graft combined with different bilaminar techniques^{3,6}. The connective tissue graft, being an autologous material, has shown more predictable results in root coverage and aesthetics and is regarded as the "gold standard"^{3,6}. However, there are some disadvantages, such as the need for two surgical areas, one for obtaining the graft and the second in the area receiving the graft, which causes greater surgical time and inconvenience to the patient during the postoperative period^{4,5}.

Given these factors, the use of bioabsorbable matrix (acellular dermal matrix) was introduced, allowing the possibility of eliminating the palate surgical donor area, thereby reducing time, patient discomfort, pain, and postoperative bleeding⁴. The acellular dermal matrix is obtained from human skin that has undergone antigen neutralization treatment; thus, it is a non-vital graft that provides architectural support and allows cellular migration to the recipient bed when surgically applied⁷.

Another alternative to using a connective tissue graft is to use enamel matrix proteins as a means to increase clinical attachment⁸. This material is composed primarily of amelogenin and proteins derived from porcine tooth buds, mimicking the epithelial root sheath⁹. Its operation is based on the periodontal structures through a similar mechanism to development of the dental follicle, promoting the apposition of new cementum on the root surface and providing greater stability to tissues inserted on that structure^{9,10}.

The objective of this study was to evaluate and compare the clinical findings obtained from treating gingival recessions with subepithelial connective tissue grafts (SECT), acellular dermal matrix (ADM), and enamel matrix proteins (EMP).

MATERIAL AND METHOD

Criteria and Groups

During the clinical research stage, 12 Miller class I and II with at least 2 mm of gingival recessions in premolar and canine without bleeding on probing were selected in patients seeking periodontal treatment in the San Francisco University School of Dentistry. The patients first underwent a training program on motivation and oral physiotherapy (to improve their oral hygiene) and were then randomly divided (by lot after the flap confection) into three groups of four patients each for the surgical procedures: subepithelial connective tissue graft (SECT), acellular dermal matrix (ADM), and enamel matrix proteins (EMP). This study was approved by the bioethics and research committee of the San Francisco of Quito University (2013-63T) and was conducted in accordance with the Declaration of Helsinki of 1995, updated in 2000. All the patients involved gave written consent to participate in our study. The following exclusion criteria were used: 1) Teeth with caries, erosions or abfraction; 2) teeth without pulpal vitality; 3) presence of inflammation on the gingival margin; 4) probing depth higher

than 3 mm; 5)smokers; 6)patients with systemic diseases or chronic users of medications; 7) pregnancy; 8) teeth with oclusal overload.

Clinical Analyses

Clinical measurements were taken before performing the surgeries by a single calibrated and standardized examiner who was not the surgeon. A periodontal probe (Hu-Friedy Chicago, IL, United States) was used for measurements and a 10k file to examine the thickness of the keratinized gingiva (Dentsply Maillefer, York, PA, United States). All measurements were made before surgery and were repeated 3 months after surgery.

The following parameters were measured: 1) Visible plaque index (VPI), 2) Bleeding on probing index (BOP), 3) Probing depth (PD), 4) Clinical attachment level (CAL): distance from the cementoenamel junction at the bottom of the gingival sulcus, 5) Height of gingival recession (REC): measure of the distance from the cementoenamel junction to the gingival margin, 6) Recession width: measured from end to the 1 mm below the cementoenamel junction in the horizontal direction, 7) Percent root coverage, 8) Keratinized tissue thickness: nominal 2 mm below the gingival margin, using a 10 file introduced in the tissue perpendicular to the bone plate to touch the periosteum; this measurement was transferred to calipers and given in millimeters, and 9) Keratinized tissue height: measure of the distance between the gingival margin to the mucogingival junction.

All measurements were made before surgery and were repeated 3 months after surgery. The plaque index and gingival bleeding index were conducted throughout the mouth.

Surgical Procedure

Surgical procedures were performed by a single surgeon with experience in the surgical techniques used.

A straight horizontal incision was made at the base of the recession, at the mesial and distal portion, using a scalpel blade 15C (Hu-Friedy, Chicago, IL, United States), supplemented by two vertical incisions. The flap was doubled in total thickness, and 3 mm of the mucogingival junction was divided to enable its coronal displacement. Manual curettes was used to planning the roots surfaces and the ethylenediamine tetra acetic acid 24% (EDTA) were applied for 1 minute to promotes the root conditioning (Figure 1).

The SECT group received a subepithelial connective tissue graft associated with coronal repositioning of the flap. The tissue was obtained from the hard palate region through a "unique" incision, where a horizontal incision was made on the palate at a distance of 2-3 mm from the gingival margin, perpendicular to the bone. Thereafter, a second incision was made from the first but at a parallel position to the long axis of the tooth, and afterwards two lateral complementary incisions were made to release the tissue. The tissue was taken from the bone with the aid of the periosteal and removed to the reception area. The grafts presented approximately 1mm of thickness while the size of the graft was defined according the size of the recessions. The graft was placed on the root of the cementoenamel junction so that it covered all the recession and 2 mm of the alveolar bone. It was fixed with sutures interrupted with absorbable thread (Vicryl, Ethicon, J&J, São José dos Campos, Brazil) (Figure 2).



Figure 1. Sequence of surgical procedure: (a) flap design; (b) protrusion of the flap; (c) smoothing the roots with manual instruments and (d) with rotary instruments.



Figure 2. Aspect of the grafts used in each group.

The ADM group received acellular dermal matrix (Puros Dermis[®] Allograft, Zimmer Dental Inc., Carlsbad, CA, United States) associated with coronal repositioning of the flap. After preparation of the reception area, the root received acellular dermal matrix hydrated in saline solution, which was previously cut to cover the root defect 2 mm apart from all sides and was fixed by points separated with absorbable suture (Figure 2).

The EMP group received a coronally advanced flap with enamel matrix proteins (Straumann[®] Emdogain, Straumann AG, Basel, Switzerland). A full thickness mucoperiosteal flap was raised by a thin periosteal. Once the mucogingival line was transferred, the dissection was partial thickness, releasing the flap from its periosteal and muscle attachment, allowing the ability to reposition the flap in a more coronal situation without any tension, and the enamel matrix derivative was applied on the root (Figure 2).

In all groups, the flap was sutured in a more coronal position to the cementoenamel junction by a suspensory suture around the tooth. The edges of the flap were sutured with simple points. The sutures were removed 10 days after surgery. In the postoperative period, the patients were oriented on conducting proper hygiene and were prescribed amoxicillin (1 g/12 hours for 7 days), rinses with 0.12% chlorhexidine digluconate (twice daily for 10 days), and 500 mg sodium dipyrone every 12 hours in case of pain.

Statistical Analysis

Due to the limited sample size of this pilot study, it was not possible to apply a normality test to assess the distribution of the data in relation to the central distribution theorem. Thus, Kruskal-Wallis nonparametric tests complemented with Dunn's test were applied for between-groups analysis, while the nonparametric Wilcoxon test was applied for within-group data analysis and to test the reproducibility of the examiner. All statistical tests were applied at the 95% confidence level. Graphpad Prism 5 software (San Diego, CA, USA) was used to perform statistical analysis of this study.

RESULT

Four patients were re-analyzed for the CAL and REC to test the reproducibility of the examiner before the surgeries. No differences between the evaluations were shown with Wilcoxon test (p>0.05). The absence of complications was verified in all groups during the operative procedure and during the postoperative period. In addition, all patients returned after three months for reevaluation. It was verified that at baseline, there were no differences in any of the parameters analyzed, demonstrating equivalence between the groups regarding clinical condition presented before the surgical procedure.

Three months after the surgical procedure, it was verified that the EMP group had lower probing depth than the SECT group (p<0.05). On the other hand, the SECT and ADM groups showed lower height and width values of the recession and improved percent values of root coverage compared to the EMP group (p<0.05). In addition, the SECT group showed greater thickness and height values of keratinized gingiva than the EMP group (p<0.05).

Regarding within-group analysis, it was verified that the SECT and ADM groups had a statistically significant reduction in the height and width parameters of the gingival recessions in the three--month period compared with the baseline (p<0.05). Table 1 shows the data as means (median) ± standard deviation of

the clinical analyses in all groups at baseline and in the 3 months after the surgical procedure. Table 2 shows the individual data of all patients before and after 3 months of the surgeries. Figure 3 exposes the clinical aspect of the gingival recessions before and after 3 months of the surgical procedures in all groups.

DISCUSSION

The enamel matrix derivative stimulates the onset of acellular cementum formation and development of the periodontal ligament and alveolar bone⁹. Some studies have shown that EMP can act in the early stages of healing, stimulating the differentiation of

Table 1. Means (median) ± standard deviation of clinical analyses in all groups at baseline and 3 months after surgical procedure

Parameter	Groups	Groups Baseline 3 months		p ^δ
Probing depth	SECT	$2.00(2.00) \pm 0.81$	$3.75(4.00) \pm 0.50^{*}$	NS
	ADM	$1.50(1.50) \pm 0.57$	$3.00(3.00) \pm 0.81$	NS
	EMP	$1.25(1.00) \pm 0.50$	$1.25(1.00) \pm 1.25^{*}$	NS
	р	NS	.03	-
	SECT	$4.75(5.00) \pm 1.25$	$3.75(4.00) \pm 0.50$	NS
Clinical attachment level	ADM	$4.75(5.00) \pm 0.50$	$3.00(3.00) \pm 0.81$	NS
	EMP	$4.25(4.00) \pm 0.50$	$2.75(2.50) \pm 1.70$	NS
	р	NS	NS	-
	SECT	$2.75(3.00) \pm 0.50$	$0.00(0.00) \pm 0.00^{*}$	<.05
Recession height	ADM	$3.25(3.00) \pm 0.50$	$0.00(0.00) \pm 0.00^{*}$	<.05
	EMP	$2.75(3.00) \pm 0.50$	$1.50(1.50) \pm 0.57^{*\#}$	NS
	р	NS	.005	-
	SECT	$3.00(3.00) \pm 0.00$	$0.00(0.00) \pm 0.00^{*}$	<.05
	ADM	$3.50(3.50) \pm 0.57$	$0.00(0.00) \pm 0.00^{*}$	<.05
Decession width	EMP	$3.00(3.00) \pm 0.81$	$2.62(2.50) \pm 1.10^{**}$	NS
Recession width	р	NS	.005	-
	SECT	$2.75(3.00) \pm 0.50$	$5.00(4.50) \pm 1.41^{*}$	NS
	ADM	$2.25(2.00) \pm 1.25$	$3.75(4.00) \pm 0.50$	NS
Kanatinina dainaina haiaht	EMP	$2.75(3.00) \pm 0.50$	$3.25(3.00) \pm 0.50^{*}$	NS
Keratinized gingiva height	р	NS	.04	-
	SECT	$0.75(0.75) \pm 0.28$	2.75(2.25) ± 1.19*	NS
	ADM	$0.62(0.50) \pm 0.25$	$1.75(1.75) \pm 0.28$	NS
Keratinized gingiva	EMP	$0.50(0.50) \pm 0.00$	$0.50(0.50) \pm 0.00^{*}$	NS
thickness	р	NS	.008	-
	SECT	-	$100.00(100.00) \pm 0.00^{*}$	-
Root coverage percentage	ADM	-	$93.75(100.00) \pm 12.50^{\#}$	-
	EMP	-	$45.84(41.67) \pm 15.67^{*\#}$	-
	р	-	.009	-

*Significant differences between SECT and EMP groups- Kruskal-Wallis c/ Dunn test. #Significant differences between ADM and DME groups- Kruskal-Wallis c/ Dunn test. δ Differences between periods within each group- Wilcoxon test. NS-No significant difference.

	Parameter/	PD		CAL		RH		RW		KGH		KGT		DCD
Patient	Patient	В	A	В	A	В	A	В	Α	В	A	В	Α	кср
SECT	1	3	4	6	4	3	0	3	0	3	4	0.5	2	100
	2	2	3	6	3	3	0	3	0	3	5	0.5	2.5	100
	3	2	4	5	4	3	0	3	0	2	4	1	2	100
	4	1	4	3	4	2	0	3	0	3	7	1	4.5	100
	5	2	4	5	4	3	0	3	0	4	4	0.5	1.5	100
ADM	6	2	3	5	3	3	0	4	0	2	4	0.5	2	100
	7	1	2	4	2	3	0	4	0	1	3	1	2	100
	8	1	3	5	3	4	0	3	0	2	4	0.5	1,5	75
	9	2	3	5	4	3	2	3	3	3	4	0.5	0.5	33.33
EMP	10	1	1	4	3	3	2	2	1.5	3	3	0.5	0.5	33.33
	11	1	0	3	1	2	1	4	4	2	3	0.5	0.5	50
	12	1	1	4	2	3	1	3	2	3	3	0.5	0.5	60.70

Table 2. Data of all the patients in all groups at baseline and 3 months after surgical procedure

B- Baseline; A-After 3 months; PD- Probing depth; CAL- Clinical attachment level; RH- Recession height; RW- Recession width; KGH- Keratinized gingiva height; KGT- Keratinized gingiva thickness; RCP- Root coverage percentage.



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Figure 3. Aspect before and after 3 months of the surgical procedures in all groups.

undifferentiated mesenchymal cells into cells producing hard tissue (bone and cementum)¹¹⁻¹³ and altering the phenotype of fibroblasts¹⁴. These cells can act selectively on exposed root surfaces, improving the degree of clinical attachment. In previous studies, similar results have been obtained to the ones verified in our study, where EMP reduced probing depth^{15,16} which may have occurred as a result of the clinical attachment gain due to regenerative potential of EMP¹⁷.

There was an increased gain in tissue height and root coverage in both SECT and ADM compared to the EMP group. This greater coverage provided by SECT and ADM may be due to the volume that these grafts presented, as EMP is a liquid state protein that does not add soft tissue to the recipient bed during the surgery. In various reviewed studies, similar to this study, the percent root coverage for ADM and SECT is quite similar^{18,19}; this result could be explained in part by the surgical protocol, as each study used the same technique in short periods of analysis.

Regarding gingival height and keratinized tissue thickness, the SECT group had a greater improvement in these parameters compared to EMP, as the biological structure is different, resulting in different healing processes. It should also be emphasized that in SECT, the recipient bed is receiving connective tissue, which provides a greater volume, while in EMP, the surgical bed receives only the enamel matrix derivative¹⁵. Another significant aspect is the phenotypic characteristics of gingival epithelium, as they are dependent on connective tissue²⁰, and for that reason, the connective tissue graft is more efficient and increases the thickness and height of keratinized tissue compared to EMP²¹. Although no significant differences regarding keratinized tissue were detected between the SECT and ADM groups, in a study with a longer time for assessment, it was shown that SECT is also more effective in obtaining keratinized tissue than ADM⁴.

This study demonstrated that ADM arises as a good alternative for treating gingival recessions, as it provides a better postoperative period for the patient and has similar results to SECT. On the other hand, EMP should be combined with SECT or ADM for the best results when clinical attachment gain is the goal. Meanwhile, this study has certain limitations, such as the limited sample size, the absence of the patients and examiner blinding, and the short assessment period. There are studies that have analyzed gingival coverage, comparing ADM and SECT, and verified that long-term healing changes the results¹⁹. Moreover, it has been suggested that the association of EMP with SECT and ADM could promote improved clinical attachment associated with the use of these grafts, as was already described in other studies that detected better clinical results when ADM²² and SECT²³ were used in conjunction with EMP compared to the sole use of these two grafts. Therefore, this hypothesis should be compared with other clinical studies because no additive effect was confirmed for EMP in other studies^{24,25}.

CONCLUSION

According to the results presented and considering the methodological limitations of this study, it can be concluded that SECT and ADM are more effective in treating gingival recessions than EMP. However, a RCT with adequate sample size and with a long time of follow up should be conduct to support these findings.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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