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MULTIPLE IMPLANT PLACEMENTS WITH SIMULTANEOUS SOFT TISSUE AUGMENTATION USING A VOLUME-STABLE COLLAGEN MATRIX COMPARED TO AUTOGENOUS CONNEVTIVE TISSUE GRAFT: A CLINICAL AND HISTOLOGICAL PILOT STUDY

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Introduction: The use of soft tissue grafts has been a key component in periodontal and implant surgeries for the past five decades, aiming to increase the width of keratinized tissue and soft tissue volume.

Objectives: This study evaluates the efficacy of a three-dimensional volume-stable collagen matrix (VCMX) compared to subepithelial connective tissue grafts (SCTG) in increasing soft tissue thickness and volume during multiple implant placements.

Methods: A randomized controlled clinical trial was conducted with four patients and eight implants. Patients were allocated to the SCTG or VCMX group, with soft tissue augmentation performed simultaneously with implant placement. Outcome measurements included soft tissue thickness, keratinized tissue height, surgical time, and histological analysis.

Results: Results indicated no statistically significant differences between the two groups over three months in keratinized mucosa height (KMH) and mucosal thickness (MT). However, the VCMX group showed reduced surgical time. Histological analysis demonstrated mature collagen and connective tissue integration in both groups.

Conclusions: Despite the small sample size, the study suggests that both SCTG and VCMX effectively improve mucosal thickness, with VCMX offering reduced surgical time and lowered morbidity and discomfort for the patient due to the absence of connective tissue harvesting from the palate.

Keywords: collagen matrix; dental implants; soft tissue augmentation; subepithelial connective tissue graft.

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The authors declare no conflicts of interest.

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POSE D'IMPLANTS MULTIPLES AVEC AUGMENTATION SIMULTANÉE DES TISSUS MOUS À L'AIDE D'UNE MATRICE DE COLLAGÈNE À VOLUME STABLE COMPARÉE À UNE GREFFE DE TISSU CONJONCTIF AUTOGÈNE: UNE ÉTUDE PILOTE CLINIQUE ET HISTOLOGIQUE

Introduction: Le recours aux greffes de tissus mous est un élément clé de la chirurgie parodontale et implantaire depuis cinq décennies, visant à augmenter la largeur et le volume des tissus kératinisés.

Objectifs: Cette étude évalue l'efficacité d'une matrice de collagène tridimensionnelle à volume stable (VCMX) par rapport aux greffes de tissu conjonctif sous-épithélial (SCTG) pour augmenter l'épaisseur et le volume des tissus mous lors de la pose de multiples implants.

Méthodes: Un essai clinique contrôlé randomisé a été mené auprès de quatre patients et de huit implants. Les patients ont été répartis dans le groupe SCTG ou VCMX, l'augmentation des tissus mous étant réalisée simultanément à la pose des implants. Les mesures des résultats comprenaient l'épaisseur des tissus mous, la hauteur des tissus kératinisés, la durée de l'intervention et l'analyse histologique.

Résultats: Les résultats n'ont montré aucune différence statistiquement significative entre les deux groupes sur trois mois en termes de hauteur de la muqueuse kératinisée (KMH) et d'épaisseur de la muqueuse (MT). Cependant, le groupe VCMX a présenté une durée opératoire réduite. L'analyse histologique a démontré une intégration du collagène mature et du tissu conjonctif dans les deux groupes.

Conclusions: Malgré la petite taille de l'échantillon, l'étude suggère que la SCTG et le VCMX améliorent efficacement l'épaisseur de la muqueuse, le VCMX offrant une réduction du temps chirurgical et une diminution de la morbidité et de l'inconfort pour le patient en raison de l'absence de prélèvement de tissu conjonctif au niveau du palais.

Mots clés: matrice de collagène; implants dentaires; augmentation des tissus mous; greffe de tissu conjonctif sous-épithélial.

Introduction

The use of soft tissue grafts has characterized the last 50 years of clinical periodontology and until today a variety of surgeries is used for clinical indications such as treatment of recessions and peri-implant soft tissue deficiencies, as well as soft tissue ridge augmentation. The use of soft tissue grafts has become a substantial element in plastic periodontal and implant surgery with two different targets being pursued: increasing the width of keratinized tissue and increasing soft tissue volume [1].

Like teeth, soft tissue stability after implant placement is a significant factor in achieving aesthetic outcomes and long-term stability. However, when teeth are extracted, the crestal ridge is covered with decreasing amounts of keratinized mucosa, which will serve as lining mucosa when dental implants are placed to restore the lost dentition. Placement of implants in insufficient gingival tissue can compromise long-term outcomes and result in soft tissue dehiscence in the implant facial aspect, a common finding following implant restorations [2]. Therefore, in these clinical situations, a soft tissue surgical augmentation procedure to increase the gingival dimensions may be recommended before, during, or after implant placement.

Both free gingival graft FGG and subepithelial connective tissue graft SCTG have been used in peri-implant soft tissue surgeries, with the palate being the most frequent donor site [3]. When the connective tissue is harvested from the palate and close to the epithelium it is more dense and stable and thus less prone to contraction, compared to the connective tissue close to the bone that contains fatty and glandular tissue [4]. Thus, the clinical procedure of graft harvesting from the palate is characterized by the challenge of obtaining an adequate and minimal amount of tissue to obtain a good graft quality while minimizing postoperative pain and reducing the risk of complications at its best [1]. This makes the procedure somehow complex, technique-sensitive and requires advanced skills and expertise. Moreover, great care is taken during harvesting a graft with the abovementioned requirements, thus increasing surgical chair time.

To reduce patient discomfort, swelling, and sometimes pain associated with the wound at the palatal donor site, soft tissue substitutes were developed and tested. It is a simple and "less traumatic" approach, which might give satisfactory results. Recently, a three-dimensionally volume-stable collagen matrix (VMCX, Geistlich Fibro-Gide®, Geistlish PharmaAG. Wolhusen.CH) was developed with promising in-vitro and experimental pre-clinical results with only a few clinical studies around dental implants [5-8]. Considering the advantage of using a biomaterial compared to the morbidity associated with connective tissue harvesting, studies are necessary to evaluate treatment indications and associated clinical benefits. This paper aims to compare the efficiency of VMCX (control) and SCTG (test) in increasing the thickness and volume of soft tissue when used simultaneously during multiple implant placements.

Null hypothesis: there is no significant difference in the efficiency of the volume-stable collagen matrix [VMCX] and subepithelial connective tissue graft (SCTG) in increasing the thickness and volume of soft tissue when used simultaneously during multiple implant placements.

Material and Methods

Study design

A pilot clinical study of 4 patients and 8 implants was conducted. The participants belonged to the pool of patients presenting at the Department of Periodontology, Faculty of Dental Medicine, Saint-Joseph University [Beirut, Lebanon] seeking treatment for multiple implant placement in the posterior mandible requiring an increase in soft tissue volume. The study was accepted and reviewed by the ethics committee of the Saint-Joseph University of Beirut (USJ -2023-62) (NCT06585813).

Inclusion criteria

1- Adults >18 years old.
2- Patients with adequate oral hygiene (FMPI<20% and FMBI<20%).
3- Healed implant sites (tooth extraction at least 8 to 12 weeks before enrolment).
4- Patients who need prosthetic rehabilitation of at least two implants in the left and/or right posterior mandibular area.
5- Inadequate amount of soft tissue where the implants are planned to be placed: thin mucosal tissues covering the edentulous alveolar ridge.
6- Compliant patients are willing to sign informed consent.

Exclusion criteria

1- Uncontrolled periodontal disease.
2- Heavy smoker (>10 cigarettes per day).
3- General contraindications for dental surgical treatment.
4- Insufficient bone volume for implant placement requiring bone augmentation procedures.
5- History of malignancy, radiotherapy, or chemotherapy for malignancy within the past 5 years.
6- Pregnancy or breastfeeding.
7- Previous and concurrent medication affecting bone and mucosal healing.
8- Disease affecting bone and connective tissue metabolism.
9- Immediate implant placement.

Clinical procedure

Following inclusion in the study, patients were scheduled for implant placement surgery with simultaneous soft tissue augmentation. Straumann® Bone Level Implants (3.3 or 4.1mm in diameter) were placed on

one mandibular side (right or left), a cover screw was placed on the implants and a concealed envelope was used to determine the allocation to one of the following groups:

A- Multiple implant placement with simultaneous SCTG (control)

B- Multiple implant placement with simultaneous VCMX (test)

In the control group, a 2-mm thickness free gingival graft [the width and length are determined by the number of implants placed] was harvested from the patient palate and then de-epithelialized. The SCTG obtained was shaped to obtain a graft of 8x8-mm width and length positioned over every placed implant, on the occlusal part of the crest in a way to cover at least 2 mm beyond the implant platform in a bucco-lingual and mesio-distal direction. The SCTG was fixed firmly to the sites using horizontal mattress sutures using 6-0 PGA sutures. Periosteal incisions were performed when necessary to ensure a tension-free closure of the site (Figure 1).

In the test group, a shaped collagen matrix with 4 mm thickness [8x8-mm width and length] was positioned over the placed implants, on the occlusal part of the crest in a way to cover at least 2 mm beyond the implant platform in a bucco-lingual and mesio-distal direction. The VCMX was fixed firmly to the sites using horizontal mattress sutures. Periosteal incisions were performed when necessary to ensure a tension-free closure of the site (Figure 2).

Patients were advised to take analgesics and anti-inflammatory medications for 3 days (Brufen 400mg; Abott) and were instructed to rinse with a 0.12% solution of chlorhexidine (Indolor; Pharmadex) twice a day for 10 days. Additionally, the patients were given 2g amoxicillin and clavulanic acid (Augmentin; GSK) per day for 7 days.

Outcome measurement

Soft tissue thickness and KT height

At implant placement [day 0], a mid-crestal incision was performed, and a flap was raised in two stages to ensure direct visibility of mucosal thickness during measurements. A full-thickness buccal flap was raised, and the thickness of the unseparated lingual flap was measured using a 1.0-mm marked periodontal probe at the bone crest at the center of the future implant sites. This procedure was repeated after 3 months of implant placement, at the second stage of surgery._Keratinized tissue height was measured before implant placement and at second-stage surgery using a 1.0-mm marked periodontal probe.

Time required for the performance of the graft

Both groups monitor and note the overall surgical time required to perform the soft tissue augmentation procedure from graft harvesting/ collagen matrix shaping until final flap fixation.

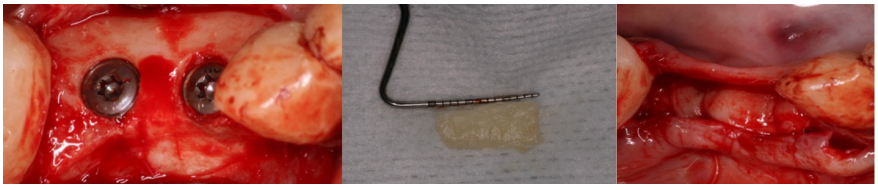

Figure 1. SCTG group. Implant placement, and stabilization on implants by horizontal mattress sutures

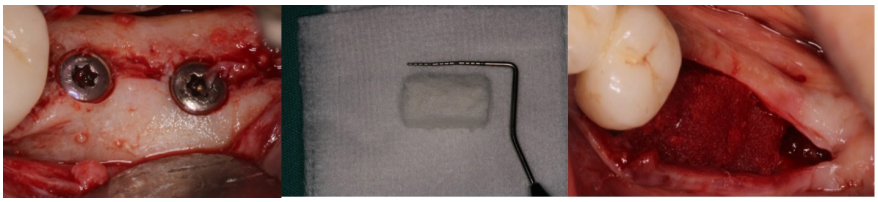

Figure 2. VCMX group. implant placement, stabilization of the Fibrogide by horizontal mattress sutures

Histological analysis

Three months post-op, during the second-stage surgery, a 3 mm punch soft tissue biopsy was obtained from the gingival tissues right above the grafted implant and was sent to histology for qualitative analysis using hematoxylin-eosin staining.

Clinical assessment

Clinical evaluation was performed on day 0/ 1 week/ 2 weeks/ 1 month/ 2 months and three months to evaluate the healing process (swelling, infection, graft exposure). and note any eventual complications.

Statistical analysis

The Wilcoxon signed-rank test, a non-parametric test, was used for within-group comparisons, and the Mann-Whitney U test for between-group comparisons. The Wilcoxon signed-rank test calculates the variations in mucosal thickness and keratinized mucosa height in both groups.

Results

Keratinized mucosa height

No statistically significant difference in KMH [Keratinized Mucosa Height] measurements between t-0 and 3 months within the CTG group was found [p-value= 0.6547]. The test statistics further suggest that the ranks of differences between the paired samples are low and do not point toward a significant change. Since all the differences between the KMH measurements at t-0 and 3 months for the VCMX group are zero, the Wilcoxon signed-rank test cannot be performed. This is because the test relies on the presence of non-zero differences to rank and compare. In practical terms, this means that there is no change in KMH measurements within the VCMX group between the two-time spots. For the CTG group, there is no significant change in KMH measurements from t-0 to 3 months based on the Wilcoxon signed-rank

test results for the VCMX group, the measurements remained the same at both time spots, indicating no change in KMH over the observed period.

These results suggest that neither the CTG nor the VCMX groups showed significant changes in KMH over the 3 months. However, the VCMX group's data specifically indicates complete stability with no variation in measurements (Table 1).

Mucosal Thickness

No statistically significant difference in MT measurements between t-0 and 3 months within the CTG group according to the Wilcoxon signed-rank test was found (p-value=0.125). Similarly, no statistically significant difference in MT measurements between t-0 and 3 months within the VCMX group according to the Wilcoxon signed-rank test was calculated (p-value=0.157). The standard deviation values suggest that the variability in MT measurements increased more in the VCMX group compared to the CTG group over the 3 months.

No statistically significant difference in MT measurements between the CTG (control) and VMCX [test] groups when combining both the t-0 and 3-month measurements was noted [p-value=0.0707]. Based on the results of the Mann-Whitney U tests, there is no statistically significant difference in MT measurements between the CTG [control] and VCMX (test) groups at any time point (t-0 or 3 months), nor when considering the combined data (Table 2).

Surgical time

The overall surgical time to perform the soft tissue augmentation procedure [from graft harvesting/collagen matrix shaping until final flap fixation] showed that VCMX was associated with reduced surgical time, as compared to the CTG group (Table 3).

Table 1. keratinized mucosa height at t0 and 3 months

Groups	Implant	KMH t-0 [mm]	KMH [mm] At 3 months	Mean evolution [mm]	p-value
CTG [control]	1	6	7	-0,25	0.718
	2	7	7		
	3	5	5		
	4	7	5		
VCMX [test]	1	4	4	0	1
	2	4	4		
	3	6	6		
	4	7	7		

Significant if p<0.05

Table 2. Mucosal thickness at t0 and 3 months

Groups	Implant	MT t-0 [mm]	MT At 3 months [mm]	Mean evolution [mm]	p-value
CTG [control]	1	1	3	1,125	0.125
	2	1	1.5		
	3	2	3		
	4	2	3		
VCMX [test]	1	1.5	1.5	1,25	0.577
	2	1.5	4		
	3	2.5	2.5		
	4	1.5mm	4mm		

Significant if p<0.05

Table 3. Mean surgical time for each group

Surgical time	VCMX	CTG
Δ[Minutes]	34	47

Histology

Biopsies in group SCTG revealed a loose network of collagen fibers with few inflammatory cells (Figure 3A). No differentiation between grafted connective tissue and the newly formed one was possible. In some specimens, some adipocytes and glandular cells were present. Vascularization was observed throughout the specimens with a relatively high number of smaller blood vessels in group VCMX, a dense collagen fiber network was present and the VCMX matrix could be identified (Figure 3B). The matrix body revealed turnover and remodeling processes. In some parts, the VCMX body was surrounded by dense connective tissue, in other parts, by a looser network of newly formed collagen fibers. Thick elastic fibers were part of the VCMX body. Vascularization was present throughout the specimens. The number of inflammatory cells was limited.

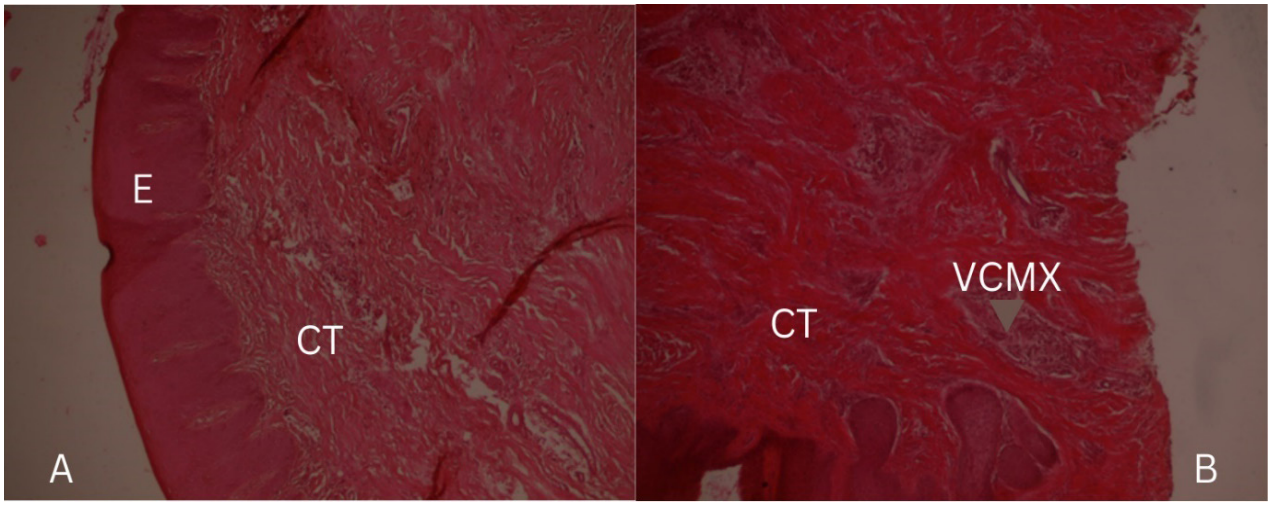

Figure 3. Histological section x10 times magnification, hematoxylin-eosin staining. A- SCTG group (Sub-epithelial connective tissue graft). B-VCMX group (volume stable collagen matrix) E-epithelium, CT-connective tissue, VCMX- collagen matrix remnants

Discussion

The results of this study indicate that while the CTG group and the VCMX group showed an augmentation in MT measurements, no group attained a statistically significant difference, and neither one of them proved superior to the other. Additionally, the variability in the measurements increased more in the VMCX group over the 3 months.

In the past, several methods and materials have been proposed to increase the volume of soft tissue before or concurrent with implant implantation, during the abutment connection, throughout the implant's healing phase, or even after the final reconstruction is inserted [9]. Clinically, the choice of the doctor, the patient's willingness to undergo the operation, and the clinical necessity all influence when and whether to augment [10]. Subepithelial connective tissue grafts [SCTGs] have been the gold standard for this purpose, even though a range of materials, including xenogeneic and allogenic materials, have been used [9].

In our study, none of the techniques proved successful in augmenting KTH. In a meta-analysis, Tavelli et al. found no statistically significant increase following any of the bilaminar techniques. Although the property of inducing keratinization of the overlying epithelium has been described as a prerogative of CTG in the natural dentition this does not seem to be the case around dental implants when CTG is used as part of a bilaminar approach [9, 10].

As with the SCTG group, we didn't find any significant difference[p-value=1] in KTH in the VCMX group between t0 and at 3 months. This fact could also be explained by the biological integration of soft tissue substitutes with the adjacent tissues. Once the blood clot has stabilized, the ingrowth of vessels into the collagen or acellular matrix subsequently leads to collagen fiber maturation[Thoma et al., 2011]. Tavelli et.al 2020 in a systematic review also reported that regardless of the substitute used in mucosal thickening no KMH variation was observed [10-12].

Clinical studies evaluating the increase in soft tissue volume following augmentation with SCTGs reported a range between 0.35–3.2 mm depending on the location and follow-up time point [7]. In this study, the increase in soft tissue volume was assessed at the level of the implant and a mean of 1.125mm increase was obtained which is in accordance with other studies. There was no statistically significant difference in soft tissue thickness gain between the SCTG and VMCX groups [p-value=0.707]. On the contrary, in another study measuring the crestal gingival thickness gain, they found a 0,5mm difference between the two groups in favor of the SCTG group, it was also confirmed in a meta-analysis [12, 13].

In our study, we found that the SCTG took 50% more time to be completed. This finding is similar to a recent study comparing VCMX with SCTG at single immediate implants, with an observed reduced surgery time for the VCMX group [14]. Another RCT didn't find any significant difference between both techniques although there was less time needed for the VCMX group [13].

The qualitative histology analysis showed at 3 months that both in the SCTG and the VCMX groups the collagen was mature, and the connective tissue turnover was apparent. A good healing pattern was noticed in the VCMX group with remnants of the collagen matrix surrounded by connective tissue. In an RCT by Thoma et al. in 2016 they found that the VCMX group had well-organized collagen fibers at 1 month and that at 2 months a dense connective tissue surrounding the VCMX remnants was visualized. Our data agrees with this finding. In another qualitative study comparing another xenogenic collagen matrix, the authors found that at 11 weeks post-tissue

transplant and stained with Hematoxylin-Eosin demonstrated the presence of numerous inflammatory cells in the connective gingival tissue at the periphery of ulceration inside of which many foreign body giant cells constitute a granuloma; similarly, newly formed collagen fibers are present, very dense and reminiscent of fibrotic tissue [15]. Also, to visualize the connective tissue and collagen heterogenicity in the VCMX group, staining with picrosirius red stain would appear to be more accurate [16].

One of the main limitations of this study is the low number of participants, due to this factor no significant results and no precise conclusion can be drawn; however, we can deduct that SCTG and VCMX both resulted in mucosal thickness improvement over multiple implants placements at the mandible. Furthermore, considering new digital technologies in assessing the volume, a consecutive volumetric study will be undertaken to get more conclusive outcomes.

Conclusion

This study found that both SCTG and VCMX effectively increased mucosal thickness over multiple implant placements in the mandible. While neither technique showed a statistically significant advantage in keratinized mucosa height or mucosal thickness, VCMX required less surgical time, and less discomfort for the patient as no connective tissue harvesting from the palate is needed, indicating a potential benefit in clinical practice. Further studies with larger sample sizes and advanced volumetric assessments are recommended to provide more definitive conclusions.

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