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Tissue integration and vascularization of volume collagen matrices and connective tissue grafts following peri-implant soft tissue augmentation: a secondary histological analysis

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Keywords

collagen-based matrix, dental implant, soft tissue augmentation, subepithelial connective tissue graft

Abstract

This study aimed to histologically reanalyze tissue integration and vascularization of volume collagen matrices (VCMX) and subepithelial connective tissue grafts (SCTG) at 3 and 4 months following peri-implant soft tissue augmentation. Peri-implant soft tissue augmentation was performed with VCMX or SCTG and biopsies were harvested at 3 and 4 months. Secondary analyses included descriptive histology, blood vessel count and tissue composition [remaining matrix, peri-implant connective tissue containing 3 different components (collagen bundles, elastic connective tissue and loose connective tissue), elastic fibers and background]. A total of 21 samples were analyzed (VCMX: 6/9 biopsies at 3/4 months; SCTG: 6 biopsies at 3 months). Compared to SCTG, VCMX sites displayed fewer and smaller blood vessels within denser tissue. Collagen bundles in VCMX were thicker, whereas SCTG sites displayed a looser tissue structure. The proportion of elastic connective tissue were 22.5% (VCMX/3 months), 32,7% (VCMX/4 months) and 17.4% (SCTG/3 months). Additionally, VCMX samples at 3 months also showed the highest proportion of elastic fibers (10,7%). Peri-implant soft tissue augmentation with VCMX resulted in reduced vascularization but increased tissue density compared to sites augmented with SCTG.

Introduction

Historically, implant dentistry has primarily focused on peri-implant hard tissue and osseointegration. More recently, greater attention has been paid to the peri-implant soft tissues, highlighting their crucial role in the long-term maintenance of peri-implant health (1,2). When soft tissues around implants are suboptimal, the most efficacious intervention to augment them is using autogenous soft tissue grafts including subepithelial connective tissue grafts (SCTG) (3,4). However, their routine use is limited by the increased morbidity and potential complications associated with the donor site (5,6).

To address these limitations, recent studies have explored alternatives such as soft tissue substitutes including volume-stable collagen matrices (VCMX). These substitutes have shown promising clinical results with reduced morbidity and similar patient-reported outcomes (5,6). Nevertheless, a notable drawback is the observed shrinkage of the augmented site (7). While peri-implant tissue health and thickness can be evaluated clinically, histological analyses are essential to prove graft integration with the surrounding tissues following soft tissue augmentation. Such analyses provide a biological basis for clinical observations, including dehiscence, shrinkage, and swelling.

Despite their importance, soft tissue biopsies are rarely performed in clinical studies, limiting a comprehensive evaluation of the biological integration of these matrices.

The aim of this study was, therefore, to histologically analyze the tissue integration and vascularization of VCMX and SCTG at 3 and 4 months following peri-implant soft tissue augmentation and to relate the findings to clinically assessed ridge volume changes.

Materials and methods

This study is a secondary histological analysis of two previous randomized controlled trials (RCT) (5,8) investigating the efficacy of VCMX (Geistlich Fibro-Gide®, Geistlich Pharma) compared to autogenous SCTG for soft tissue volume augmentation at implant sites. In the first RCT, biopsies were collected at the 3-month follow-up (5), while in the second RCT, biopsies were obtained at the 4-month follow-up (8). Both studies received ethical approval, including permission to obtain biopsies, from the Ethical Commission of Canton Zurich (Kantonale Ethik-Kommission Zürich, ref. numbers KEK-ZH-Nr. 2011-0408 and KEK-ZH-Nr. 2013-0398).

At 3 (RCT 1)(5) and 4 months (RCT 2)(8) of follow-up, a minimally invasive abutment connection was performed using a U-shaped incision design on the crest with a microsurgical blade (Swann-Morton, Sheffield, UK). The cover screw of the implant was removed and a small biopsy (roughly 2x2mm) of the soft tissue on top of the implant was harvested. The tissues were fixed in formaldehyde, dehydrated in alcohol, immersed in xylol as an intermedium and finally embedded in paraffin. Embedded samples were cut with a 5 microns-thickness using a microtome (Medite Service AG, Dietlikon, Switzerland). Two sections per block were prepared and stained with either Hematoxylin Eosin (HE) or Van Gieson-Elastica (VG-El) staining. All histological sections were evaluated using a light microscope (Leica DM6 M, Leica Microsystems, Wetzlar, Germany).

For each sample, a defined region of interest (ROI) was chosen in the centre of the connective tissue whenever possible, and all measurements were performed within this area. Blood vessel counts were conducted using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The detailed image processing workflow for the quantitative histomorphometric analysis is described in Fig. 1. This analysis identified the remaining matrix and the different components of the connective tissue, as described in previous studies (9,10). First, the digitized original VG-El-stained images of the regions of interest (Fig. 1. A) were processed using an image-processing program (Adobe Photoshop, San Jose, CA, USA) to reduce the number of tones (Fig. 1. B). Then, the different tissue components (remaining matrix, peri-implant connective tissue containing three components (collagen bundles, elastic connective tissue and loose connective tissue), elastic fibers and background) were manually selected and identified using distinct colors (Fig. 1. C-D). Finally, this last image was analyzed using the microscope image analysis software and the proportion of the different tissue components was calculated with the same software (LAS X, Leica Microsystems) (Fig. 1. E). One masked, experienced histologist performed all the image processing and the corresponding measurements (SH). Descriptive statistical analyses of the metric variables were performed by calculating the means, standard deviations and medians. ANOVA followed by a post hoc Tukey test was used to compare the different groups.

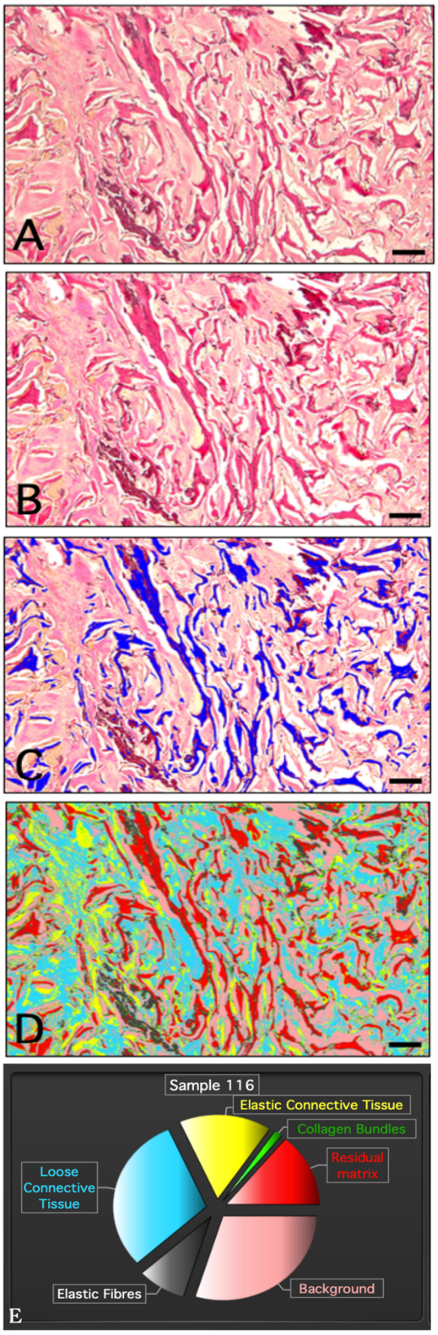

Figure 1. Image processing of a representative sample VCMX for the quantitative histomorphometry analysis. Steps B to D were performed using Adobe Photoshop software. **A**, Original image of the region of interest (Van Gieson Elastica staining) taken with a light microscope. Scale: 100 µm; **B**, Image processing to reduce the number of tones and facilitate the observation of the different tissues; **C**, Manual selection of the collagen bundles (areas selected in blue); **D**, Manual selection of all the different tissues; **E**, Tissue quantification was performed on Image D using LAS X software.

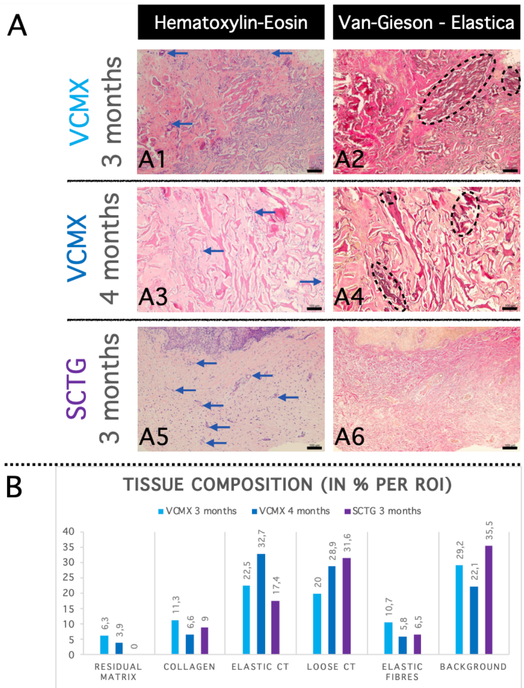

Figure 2: Descriptive histology and quantitative histomorphometry. **A**, Descriptive histology using Hematoxylin Eosin (A1,3,5) and Van Gieson Elastica (A2,4,6) staining. Blood vessels are shown using blue arrows. Magnification x100. Scale bar: 100 micrometers. Elastic fibers from the matrix are surrounded by dotted lines. A1, A2, VCMX at 3 months; A3, A4, VCMX at 4 months; A5, A6, SCTG at 3 months. **B**, Tissue composition resulting from the quantitative histomorphometry analysis. Abbreviations: CT, connective tissue; ROI, region of interest, SCTG, sub epithelial connective tissue graft; VCMX, volume collagen matrix.

Results

In total, 21 samples were analyzed: 6 VCMX and 6 SCTG biopsies at 3 months and 9 VCMX biopsies at 4 months. Since the tissue integration of SCTG was complete and optimal at 3 months (n=6), only VCMX samples were analyzed at 4 months (n=9). On the VCMX samples at 3 months (n=6), residuals of the matrix were observed, mainly composed of collagen bundles associated with dense packages of elastic fibers (Fig. 2. A1- A2). The surrounding tissue was denser, displaying a higher amount of collagen as well as a lower amount and smaller blood vessels in comparison to native gingival connective tissue and tissue augmented with SCTG (n=6). At 4 months, samples grafted with VCMX (n=9) displayed matrix remnants of a smaller size, well integrated into the surrounding tissue (Fig. 2. A3-A4). At 3 months, the SCTG perfectly integrated into the surrounding tissue, resulting in a homogenous connective tissue with a low amount of thin elastic fibers (Fig. 2. A5-A6). The specific analysis using Photoshop enabled us to distinguish the collagen bundles of the matrix from the ones of the native connective tissue as they display different densities, resulting in different tones (Fig. 1).

Concerning tissue composition, similarly to descriptive histology, VCMX samples were more compact, while STCG samples displayed more loose connective tissue and more background (Table 1 & Fig. 2. B). At 4 months, the residual matrix in VCMX samples was approximately half the amount observed at 3 months (Table 1 & Fig. 2. B). This reduction was accompanied by a decrease in collagen bundles and a simultaneous increase in elastic connective tissue. The increase in elastic connective tissue in VCMX samples at 4 months was statistically significant compared to SCTG samples at 3 months (Table 1 & Fig. 2. B).

Table 1. Vascularization and tissue composition of the retrieved biopsy samples.

		VCMX 3 months	**VCMX 4 months**	**SCTG 3 months**	**p-value**
Vascularization	**Number of vessel lumen (per ROI)**				
	Mean ± SD	11.9 ± 19.6	10.0 ± 4.1	15.1 ± 8.0	p=0.178
	Median	8.0	10.0	12.5	
Tissue composition	**Matrix** (% per ROI)				
	Mean ± SD	6.3 ± 7.0	3.8 ± 4.5	0 ± 0	p=0.098
	Median	4.8	2.8	0	
	Collagen bundles (% per ROI)				
	Mean ± SD	11.3 ± 6.3	6.58 ± 5.30	9.1 ± 10.3	p=0.479
	Median	11.6	5.1	3.8	
	Elastic connective tissue (% per ROI)				
	Mean ± SD	22.4 ± 9.7	32.7* ± 11.4	17.3* ± 9.1	**p=0.030**
	Median	19.7	31.0	17.8	
	Loose connective tissue (% per ROI)				
	Mean ± SD	20.0 ± 11.7	28.8 ± 8.2	31.5 ± 8.2	p=0.104
	Median	17.0	29.9	34.2	
	Elastic fibres (% per ROI)				
	Mean ± SD	10.7 ± 7.6	5.7 ± 3.9	6.5 ± 5.9	p=0.264
	Median	7.9	6.5	4.9	
	Background (% per ROI)				
	Mean ± SD	29.1 ± 14.3	22.1 ± 9.4	35.5 ± 12.7	p=0.129
	Median	26.1	19.2	31.8	

Note. Performed statistical analyses were Anova (Fisher test) and post hoc Tukey test. * <0.05.
Abbreviations: ROI, region of interest; SCTG, subepithelial connective tissue graft; SD, standard deviation; VCMX, volume collagen matrix.

Discussion

The present study evaluating tissue integration and vascularization of volume collagen matrices (VCMX) and subepithelial connective tissue grafts (SCTG) at 3 months following peri-implant soft tissue augmentation predominantly revealed:

> (i) VCMX-augmented sites displayed fewer blood vessels compared to SCTG, with smaller diameters, situated within denser connective tissues containing increased amounts of elastic connective tissue and residual matrix material
>
> (ii) SCTG-augmented sites exhibited a greater number of thicker blood vessels and a looser connective tissue structure. Moreover, the integration of SCTG was considered optimal as the grafted tissue could no longer be distinctly identified from the surrounding native tissue

Histology remains the gold standard for characterizing and understanding tissue dynamics. According to the manufacturer, histological staining identified a considerable proportion of thick elastic fibers and collagen fibers in VCMX. This finding is in line with VCMX's composition, which includes crosslinked collagen and elastin. Interestingly, while elastic fibers derived from the matrix (elastin) may appear similar in color to elastic fibers found in native gingival connective tissue (mainly oxytalan and elaunin), their greater thickness and distinct location suggest a different remodeling process.

Previous studies have also shown VCMX rapid colonization by cells and blood vessels, which is facilitated by its large pore size, density and interconnected structure (11). Taken together, these findings support the hypothesis that volume loss in VCMX-augmented tissues could be attributed to remodeling by fibroblasts occurring more rapidly than tissue synthesis (11,12). This imbalance may partly explain the increased shrinkage observed with VCMX compared to SCTG (5). Notably, the residual matrix volume in VCMX samples decreased by nearly half between 3 and 4 months, consistent with volume analyses reported in other studies (7,13).

Correlating histology to clinical findings can provide some hints for optimizing tissue stability and treatment efficacy. This is clinically relevant as soft tissue substitutes are increasingly used to reduce patient morbidity for soft augmentations at implant sites, despite their seemingly lower clinical efficacy compared to autogenous grafts (5,6). In addition, recent evidence suggests that VCMX can still achieve soft tissue augmentation at implant sites, even in patients with reduced vascularization and impaired healing capacity, such as smokers (14).

The precise underlying mechanism dictating VCMX shrinkage over time remains unclear, but it might be linked to a significantly higher proportion of elastic connective tissue. The main limitation of this study was the sample size. In cases where soft tissue augmentation was insufficient, biopsy size had to be reduced, making the analysis impossible. This variability in harvested samples was largely due to the greater tissue shrinkage observed in the VCMX group, which limited biopsy size to avoid compromising the clinical outcome of the augmentation procedure.

To gain a deeper understanding of VCMX remodeling and shrinkage, further clinical studies with larger sample sizes and standardized tissue sampling at multiple time points are needed. Tracking the changes in the composition of augmented tissue during healing could help understand the clinically observed tissue contraction, particularly within the first months. However, such sampling should only be performed when it does not jeopardize the clinical success of the augmentation procedure.

Conclusions

Peri-implant soft tissue augmentation with VCMX resulted in reduced vascularization, but a greater tissue density compared to sites augmented with SCTG.

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