

Histological evaluation of subepithelial connective tissue grafts harvested by two different techniques. Preliminary study in humans

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ABSTRACT

Subepithelial connective tissue graft (SCTG) is an essential therapeutic tool in periodontal plastic surgery and implantology. The aim of this preliminary study was to observe and make a histological and histomorphometric comparison of the composition of subepithelial connective tissue grafts (SCTGs) harvested from the palatal mucosa by two different harvesting techniques: mucoperiosteal (lamina propria and complete submucosa including periosteum) and mucosal (lamina propria and a portion of the submucosa). The main hypothesis proposes that SCTG harvested with the mucosal technique contains a greater proportion of connective tissue proper (CTP) and a lower proportion of adipose tissue (AT) than the mucoperiosteal technique.

Twenty healthy patients who required SCTG for different purposes were selected and assigned to one of the two following groups: group A (n=10; mucoperiosteal harvesting technique) and group B (n=10, mucosal harvesting technique). The histological sample was obtained by removing a 2 mm thick slice from the most distal portion of the graft. The proportions

of adipose tissue (AT), connective tissue proper (CTP) and vascular tissue (VT) were evaluated.

In group A, histomorphometric analysis showed that CTP accounted for 58.2% of the graft while AT accounted for 32.64%. In group B, the proportions of CTP and AT were 79.86% and 11.93%, respectively. The differences between groups were statistically significant for both tissues ($p < .05$). In contrast, no statistically significant difference was observed in the proportion of VT. Within the limitations of this study, the results show that the SCTGs harvested by the mucosal technique contain a greater proportion of CTP and a lower proportion of AT than those obtained by the mucoperiosteal technique, whereas the proportion of VT does not differ.

Further long-term clinical and histological studies with more samples are needed to evaluate the clinical implications of SCTG composition.

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Keywords: Periodontics; Grafts; Histology.

Evaluación histológica de los injertos de tejido conectivo subepitelial del paladar mediante dos técnicas diferentes de obtención. Estudio preliminar en humanos

RESUMEN

El injerto de tejido conectivo subepitelial (ITCSE) es una herramienta indispensable en la cirugía plástica periodontal y la implantología.

El objetivo del presente estudio preliminar fue observar y comparar histológica e histomorfométricamente la composición de los injertos de tejido conectivo subepitelial (ITCSE) obtenidos de la mucosa palatina mediante dos técnicas diferentes: mucoperiostica (lamina propia y submucosa incluyendo el periostio) y mucosa (lámina propia y parte de la submucosa). La principal hipótesis postula que el ITCSE obtenido mediante la técnica mucosa contiene mayor proporción de tejido conectivo propiamente dicho (TCP) y menor proporción de tejido adiposo (TA) que el obtenido mediante la técnica mucoperiostica.

El presente estudio incluyó veinte pacientes sanos que requerían ITCSE por diferentes motivos, los cuales fueron distribuidos de forma equitativa en dos grupos: grupo A (n=10; técnica de obtención mucoperiostica) y grupo B (n=10; técnica de obtención mucosa). La muestra histológica se obtuvo removiendo una

porción de 2 mm de ancho de la parte más distal del injerto. Se evaluó la proporción (%) de tejido adiposo (TA), tejido conectivo propiamente dicho (TCP) y tejido vascular (TV).

En el grupo A, el análisis histomorfométrico mostró que el TCP constituía el 58.2% del tejido mientras que el tejido adiposo constituía el 32.64%. En el grupo B, la proporción de TCP y AT fue 79.86% y 11.93%, respectivamente. Las diferencias observadas entre los grupos fueron estadísticamente significativas para ambos tejidos ($p < .05$). En cambio, no se observaron diferencias estadísticamente significativas en la proporción de TV. Dentro de las limitaciones del presente estudio, los resultados mostraron que los ITCSE obtenidos mediante la técnica mucosa contienen mayor proporción de TCP y menor proporción de TA que los obtenidos con la técnica mucoperiostica, mientras que el TV permanece estable.

Se requieren estudios longitudinales clínicos e histológicos a largo plazo con mayor cantidad de muestras para evaluar las implicancias clínicas de la composición del ITCSE.

Palabras clave: Periodoncia; Injertos; Histología.

INTRODUCTION

Subepithelial connective tissue graft (SCTG) is currently considered an essential therapeutic tool in periodontal plastic surgery and implantology.¹⁻³

It was initially used to increase the volume of the edentulous ridge and the width of keratinized gingiva.^{4,5} Subsequently, it was used for numerous procedures such as root coverage,⁶⁻¹³ soft tissue augmentation around dental implants and partially edentulous areas,^{2,3,14-16} papilla reconstruction, and scar correction.¹⁷⁻¹⁹

SCTG has shown better aesthetic and biological behavior outcomes in different procedures than have other treatments such as free gingival grafts, allografts, and guided tissue regeneration.^{1-3,20}

In order to minimize surgical trauma and reduce post-surgical discomfort, many authors have proposed different harvesting techniques.^{6,7,9-12,21-24}

They can be classified into two groups: mucoperiosteal techniques (lamina propria and complete submucosa including periosteum),^{6,9,21,22} and mucosal techniques (lamina propria and a portion of the submucosa),^{10,23,24}. Mucosal techniques have better post-surgical evolution at the palatal donor site, since the periosteal portion remains mostly attached to the bone plate, acting as a protective barrier and a source of vessels (supra-periosteal vessels), reducing wound healing time and patient morbidity.^{10,23}

In the mucoperiosteal techniques, the deep portion of the submucosa, which is mainly composed of adipose and/or glandular tissue, is always included in the graft. Some authors²⁵ suggest that this tissue should be removed because it can interfere with the revascularization of the graft and it may work "as a barrier both to diffusion and vascularization".

Ouhayoun et al.²⁶ performed a histological and biochemical analysis of SCTG human samples and suggested that the deep portion of the connective tissue from the palate could not induce keratinization. In the mucosal techniques, the graft can be obtained with a double-bladed scalpel which has parallel blades set 1.5 mm apart.^{10,23,24} This technique enables a graft of homogeneous thickness to be obtained and the deep portion of the submucosa, which remains attached to the osseous plate, to be excluded.

Differences in the composition of the graft obtained by the mucosal technique (with a double-blade scalpel) were found in a human histological study.²⁷

While some samples consisted almost exclusively of lamina propria, others contained higher proportion of submucosa, which is mainly composed of adipose tissue. The authors observed that in all cases, the submucosa lies deeper to the lamina propria. This implies that if a thicker graft is taken by extending the dissection deeper, there is an increase only in the amount of submucosa, while the amount of lamina propria remains constant. It was also observed that the resulting portion of lamina propria could be greatly variable, ranging from 21.1% to 100% of the total composition of the graft. The results of this study suggest that grafts harvested from more superficial areas (closer to the epithelium) would increase the proportion of lamina propria within the graft. This approach, however, will increase the risk of including epithelium in the graft.

To date, no human study has been carried out comparing mucoperiosteal versus mucosal harvesting techniques. Therefore, the aim of the present preliminary study was to describe and compare – through histological and histomorphometric analysis – the composition of SCTG harvested from the palatal mucosa by a modification of the single incision technique (mucoperiosteal)²¹ and a modification of the double-bladed scalpel technique (mucosal).^{23,24}

The main hypothesis of this study proposes that SCTG harvested with the mucosal technique, excluding the deep portion of submucosa, contains a greater proportion of connective tissue proper and a lower proportion of adipose tissue than the SCTG harvested by the mucoperiosteal technique.

MATERIALS AND METHODS

Patient selection

Twenty patients who were referred to the Department of Periodontology, School of Dentistry, University of Buenos Aires (FOUBA), with procedures requiring SCTG for different purposes, were assigned to two groups according to their need for treatment: Group A: SCTG harvested through mucoperiosteal procedure,²¹ in patients who mainly required ridge augmentations (n = 10), and Group B: SCTG harvested by mucosal procedure,^{23,24} in patients who mainly needed root coverage procedures (n=10).

Average patient age was 41.5 years (18-65). All patients had good general health. Smokers, patients

with uncontrolled systemic diseases and anticoagulated patients were excluded from the study.

All patients in the study accepted the clinical procedures and signed informed consent approved by the FOUBA Ethics Committee (No. 029/14).

Surgical procedure: sample collection

All grafts were harvested by one experienced periodontist under magnified vision (10x) using an operative microscope (Zeiss ST, Carl Zeiss, Feldbach, Switzerland), from the palatal area comprised from canine to the first molar.

In both groups, the original technique^{21,23} was modified: after the initial incision and before taking the graft, a full thickness detachment was performed up to 3 mm from the incision. This limited flap elevation was performed in both groups to allow better access and positioning of the blade, enabling the superficial incision to be placed parallel to the surface of the mucosa, avoiding the concave surface of the palate.²⁴

The superficial incision was placed approximately 1 mm away from the epithelial surface, in order to avoid the inclusion of epithelium in the graft. The graft was harvested with a conventional scalpel in the mucoperiosteal (total thickness) technique and with a double-bladed scalpel in the mucosal (partial thickness) technique. Immediately after obtaining the SCTG, it was placed on a wooden tongue depressor without losing the reference of its original location in the palatal mucosa. The histological sample was obtained by removing a 2 mm thick

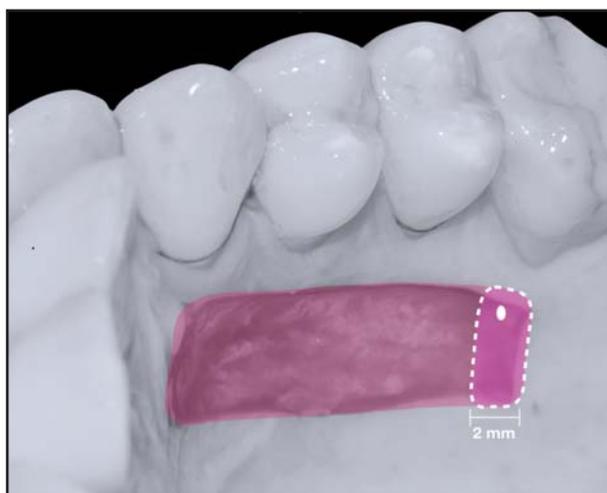


Fig. 1: Area of the subepithelial connective tissue graft is marked on a clinical image. A 2mm distal portion was used for histological evaluation. The white point represents the suture performed to indicate sample orientation (dotted line).

slice from the most distal portion of the graft. A suture (Prolene 6-0 p1 Ethicon, Johnson & Johnson, Somerville, NJ, USA) was placed in the most coronal and superior portion of the graft (where the initial incision was made), which identifies the side and orientation of the sample (Fig. 1). All samples were immediately fixed in 10% formalin for histological analysis.

Histological processing

The length, width and thickness of the macroscopic sample was measured with a Vernier caliper. The samples were histologically processed to obtain 7 mm thick longitudinal sections, which were stained with hematoxylin-eosin and Masson's trichrome. The sections were observed under light microscope.

Histological and histomorphometric analysis

All the sections were first scanned with a microscope (AXIO lab a1 Carl zZeiss) at 5x magnification at a resolution of 0.321 $\mu\text{m}/\text{pixel}$, by a digital virtual microscopy system (Carl Zeiss Zen Blue edition 2011). The most representative section was selected and digital JPEG images were obtained. Finally, tissue composition was analyzed with image analysis software (Image Pro Plus).

Histomorphometric analysis was performed by one of the authors, who was blinded regarding which harvesting technique had been used to obtain the graft. The following parameters were delimited on the microphotographs:

- CTP/TA (%): Connective tissue proper area = fraction of total area corresponding to connective tissue proper area
- AT/TA (%): Adipose tissue area = fraction of total area corresponding to adipose tissue area
- VT/TA (%): Vascular tissue area = fraction of total area corresponding to vascular tissue area

Total area (TA) value was the measurement of the area of the whole histological section. ($\text{TA} = \text{CTP} + \text{AT} + \text{VT}$).

Statistical analysis

Quantitative variables were described by Mean (M), standard deviation (SD) minimum (MIN) and maximum (MAX).

A grouped t-test was used to compare the percent of adipose tissue (AT), vascular tissue (VT) and connective tissue proper (CTP) between the two groups.

The data met the conditions of normality and homoscedasticity required for performing grouped Student's t-test. The assumption of normality was analyzed by the Shapiro-Wilk test with modifications. Homoscedasticity was analyzed by F test for equality of variances. The statistical value (t), the degrees of freedom (df) and the p-value were reported. A statistically significant result was considered when the p-value was less than .05. The 2016 version Infostat program was used.²⁸

RESULTS

The results of the histomorphometric analysis, regarding the relative proportion of CTP, AT and VT in CTG harvested with mucoperiosteal and mucosal techniques are summarized in Table 1.

In group A (mucoperiosteal technique) the mean value for AT was 32.64%, VT was 8.05% and CTP accounted for 58.52% of the graft. In most samples, the grafts consisted of two different parts: the most superficial portion with dense connective tissue and the deep portion with adipose tissue (Figs. 2, 3). In others samples, the SCTG was almost entirely composed of submucosal tissue. Glandular tissue (GT, minor salivary mucosal glands) was present in one sample in Group A (7.64%; Fig. 4).

In group B (mucosal technique) the mean value for AT was 11.93%, VT was 8.03%, and CTP accounted for 79.86% of the graft. In some cases, the graft was mainly composed of connective tissue proper with zones of extremely dense collagen fibers (Fig.5). In other cases, the density of collagen fibers was moderate, with areas of loose connective tissue (Fig. 6). Epithelium (E) was present in two samples in Group B (1.18%, 0.57%; Fig. 6).

Statistically significant differences were found in the composition of the grafts according to harvesting technique. A higher proportion of CTP

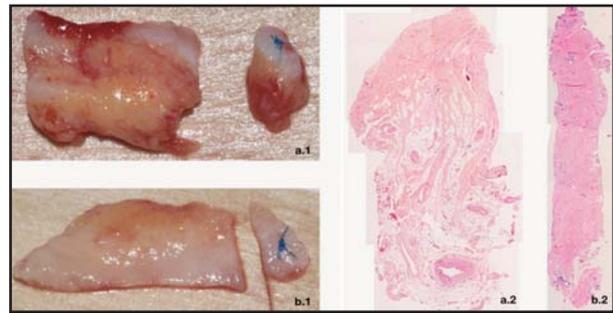


Fig. 2: Longitudinal sections of the grafts were obtained and stained with hematoxylin-eosin (H-E): Group A (mucoperiosteal technique). a.1: Sample collection. a.2: Longitudinal section (H-E stain, original magnification X5). Group B (mucosal technique). b.1: Sample collection. b.2: Longitudinal section (H-E stain, original magnification X5).

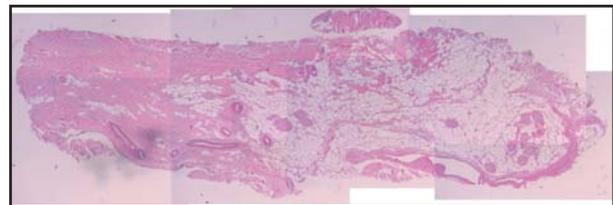


Fig. 3: Histological appearance of mucoperiosteal graft: (H-E stain, original magnification X5). A great proportion of adipose tissue and an increased diameter of vascular vessels are observed in the deeper area.

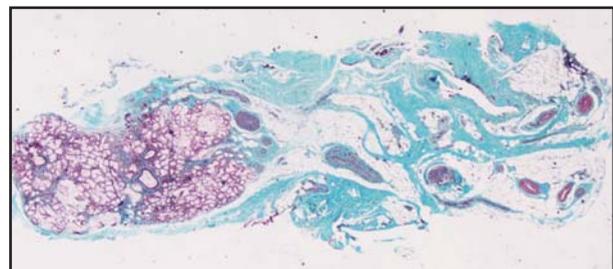


Fig. 4: Histological appearance of mucoperiosteal graft in which a minor salivary gland is observed (GT) (Masson's stain, original magnification X5).

Table 1: Composition of the grafts: Mucoperiosteal versus mucosal technique.

Component	Group A: mucoperiosteal technique (n=10)				Group B: mucosal technique (n=10)				Grouped t-test		
	Mean	SD	MIN	MAX	Mean	SD	MIN	MAX	T	df	p
CTP (% of TA)	58.52	9.78	45.71	78.5	79.86	10.11	63.34	92.47	3.6	9	0.003*
AT (% of TA)	32.64	10.2	12.36	47.38	11.93	8.43	0.97	31.12	-3.74	9	0.002*
VT (% of TA)	8.05	1.54	4.98	9.97	8.03	5.25	3.28	21.45	-.02	9	0.494

*Statistically significant difference, $p < .05$

AT= adipose tissue; CTP= connective tissue proper; df= degrees of freedom; MAX= maximum MIN= minimum; SD= standard deviation; T= statistical value; TA= total area; VT= vascular tissue.

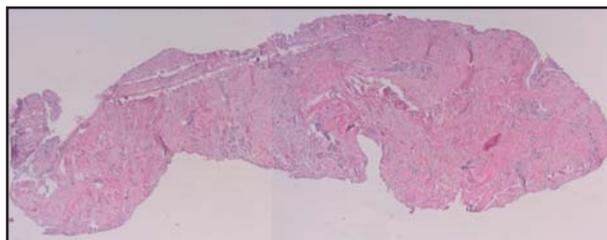


Fig. 5: Histological appearance of mucosal graft: graft composed only of lamina propria. (H-E stain, original magnification X5).

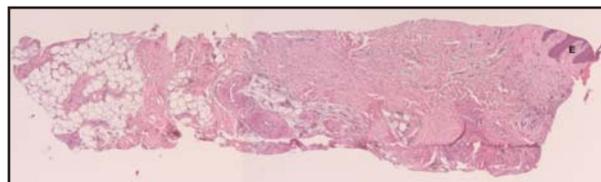


Fig. 6: Histological appearance of mucosal graft: Minimal epithelial tissue (E) and dense connective tissue can be observed in the most superficial portion. (H-E stain, original magnification X5).

and a lower proportion of AT were found in the mucosal technique than in the mucoperiosteal technique ($p = .003$ and $.002$, respectively).

No statistically significant difference was found between groups for the proportion of VT ($p > .05$).

DISCUSSION

Subepithelial connective tissue grafts are widely used in periodontal and peri-implant plastic surgery.¹⁻³ Although many harvesting techniques and modifications have been proposed since Edel in 1975,^{4,6,7,9-12,21-24} there are few studies that describe the histological composition of the tissue harvested from humans.^{26,27}

One recent histological study in fresh human cadavers²⁹ showed that the harvesting technique is important in the composition of SCTG. The authors concluded that SCTG harvested with de-epithelialized technique contains higher proportions of dense connective tissue and lower proportions of adipose tissue than SCTG harvested with split-flap technique (deeper area).

To date, there is no study in humans comparing the composition of the grafts harvested with two different “subepithelial connective tissue graft” techniques; i.e., without removing epithelium with the graft (such as free gingival graft or de-epithelialized free gingival graft). The aim of the present preliminary human study was to describe and compare the histological and histomorphometric characteristics of SCTG harvested from the palatal mucosa by two modified techniques: the single incision technique (mucoperiosteal – total thickness– technique)²¹ and the double-bladed scalpel technique (mucosal –partial thickness– technique),^{23,24} to evaluate whether the harvesting technique is an important factor in the relative composition of adipose tissue/connective tissue proper.

The palatal mucosa is composed of an epithelial layer, a lamina propria, a submucosa and the periosteum. The thickness of these tissues is relatively uniform for the epithelium and the lamina propria and highly variable for the submucosa.¹¹ The epithelial layer in the human palatal mucosa is about 0.5 mm thick and the lamina propria is about 1 mm.³⁰ The limited amount of lamina propria poses a surgical challenge when obtaining a subepithelial graft composed of collagen-rich connective tissue with the standard techniques. The main limitation lies in the curved shape of the palatal mucosa, which makes difficult to maintain the superficial incision close to the epithelial surface, especially in the most apical areas. For this reason, a modification of the standard technique was performed in this study, by elevating 3 mm full thickness mucosa in order to facilitate access with the blade.²⁴

In the current study, the presence of lamina propria (connective tissue proper) was observed in both modified techniques. The main histological difference between the techniques was the amount of adipose tissue content, with 20.14 % for the mucosal vs. 41.48% for the mucoperiosteal technique, and the amount of connective tissue proper with 79.86% and 58.52%, for the mucosal and mucoperiosteal technique, respectively ($p < .05$). Although previous studies^{25,26} have suggested that this tissue may not induce keratinization and may interfere with the revascularization of the graft, its clinical relevance has not yet been confirmed.

On the other hand, the amount of vascular tissue was similar in both groups ($p < .05$), although wider vessels were observed only in the deep submucosa of the mucoperiosteal group, which may partly explain the increase in bleeding observed while this technique is being performed. Glandular tissue was

found only in 1 case, representing 7.64% of the graft, while epithelium was present in two samples in the mucoperiosteal group in minimal proportions (1.18%, 0.57%) but was not found in the mucosal technique group. This could be important considering that, although some authors have suggested that the inclusion of epithelium in the graft does not affect clinical results;^{6,9,27} others have reported complications as a result of epithelial cysts and edema.³¹

FUNDING

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