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Randomised controlled trial

XENOGENEIC DERMAL MATRIX *VERSUS* AUTOLOGOUS CONNECTIVE TISSUE GRAFT *VERSUS* NO GRAFT AT ABUTMENT CONNECTION FOR IMPROVING AESTHETICS: 6-MONTH OUTCOMES OF A RANDOMISED CONTROLLED TRIAL

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OBJECTIVES. The aim of this multicentre randomised controlled trial was to evaluate the efficacy of a xenogeneic dermal matrix in widening keratinised peri-implant tissues during second-stage surgery, and to compare it to both autologous connective tissue graft and a control group with no augmentation.

MATERIAL AND METHODS. Patients requiring an increase in keratinised gingiva width were enrolled by four university/dental practices and randomised into three different groups for grafting procedures at the implant uncovering stage: either xenogeneic dermal matrix (Group X), autologous connective tissue graft (Group A) or no graft (control, Group C). The primary outcomes were width of keratinised tissue and facial soft tissue levels, evaluated at three different time points (T0, implant uncovering stage; T1 and T2, six weeks and six months after surgery, respectively). Secondary outcomes were: implant failure, complications, marginal bone loss, papilla index, facial soft tissue level, pink esthetic score, and aesthetic assessment by patients.

RESULTS. Thirty-six patients, with one implant per patient, were enrolled at two centres (18 at each centre): 12 for control, 12 for xenogeneic dermal matrix and 12 for autologous tissue graft. Three patients dropped out and two patients from the autologous group had implant failures. No complications were recorded. After six months, the width of keratinised tissue increased by 0.16 ± 1.01 (P = 0.79), 1.05 ± 0.76 (P = 0.01) and 0.80 ± 1.73 mm (P = 0.28), and facial soft tissue level was -0.95 ± 0.85 (P = 0.04), 0.32 ± 0.57 (P = 0.15) and 0.35 ± 0.79 mm (P = 0.30) respectively in Groups C, X and A groups. Between-group analysis showed that, with respect to control, only facial soft tissue level (1.31 mm, P = 0.01) and width of keratinised mucosa (2.43 mm, P = 0.01) outcomes in the autologous graft group were statistically significant at T2. Mean marginal bone loss between T0-T2 was -0.4 ± 0.4mm, with no differences between groups. Pink aesthetic score showed no significant differences between groups, being 0.89 for A-C (P = 0.41), 0.88 for A–X (P = 0.63) and 0.72 for X-C (P = 0.88).
Patient's aesthetic satisfaction (Visual Analogue Scale) was 92.2 ± 8.4, 93.8 ± 7.7, 97.2 ± 3.0, for Groups C, X and A, respectively. Between the two dental centres, only facial soft tissue level at T0–T2 was significantly different, by 0.67 ± 0.62 mm (P = 0.03).

CONCLUSIONS. After six months, autologous connective tissue graft yielded a significant gain in facial soft tissue levels and width of keratinised mucosa, as compared to the control group (no graft).

CONFLICTS OF INTEREST STATEMENT. This study received support from Tecnoss, which provided Derma membranes, and Sweden & Martina, which that provided free dental implants and healing abutments.
Dr. Barone has received speaker fees from Tecnoss. No other authors have any conflict of interest to declare.

INTRODUCTION

In single-tooth implant restorations, the final level of the gingival margin depends on multiple factors such as the peri-implant biotype and crestal bone level, as well as the corono-apical position and inclination of the implant[1]. Peri-implant soft tissue recession is a common finding[2]. It usually develops during the first six months after prosthetic loading, mainly on the buccal side, and results in exposure of the metal component, thereby compromising the aesthetics of the restoration[2,3]. Aesthetic failure can also be caused by mismatching soft tissue colour and texture, and incomplete/absent interdental papillae[4]. In addition, the colour of the implant metal is more likely to be visible through a thin peri-implant mucosa, impairing the aesthetic outcome[5].

Linkevicius et al. detected a correlation between clinical outcome and gingival thickness, with less bone loss around implants surrounded by thick mucosa (≥ 2 mm)[6]. On the other hand, in a 5-year prospective clinical trial, Todisco et al. recorded that, although the height of the keratinised mucosa did not seem to alter the clinical outcomes, its presence on vestibular and lingual sites was associated with increased marginal bone loss when compared to implants having at least one side without keratinised mucosa[7].

In fact, even if the presence of keratinised peri-implant mucosa does not seem to alter long-term implant survival, it can influence the biological outcome[8]. Lang & Loe suggested that a width of at least 2 mm of keratinised tissue is essential for peri-implant health[9]. This has been confirmed by studies showing that implants surrounded by less than 2 mm of keratinised tissue are more likely to accumulate plaque, have a 3-fold greater chance of developing bleeding on probing, and are more prone to cause discomfort during brushing[10,11].

The volume of keratinised peri-implant tissue can be increased via different augmentation techniques, which can be performed as a preliminary intervention, during implant placement, at the uncovering phase, or at any time gingival recession appears[12]. At present, however, there is insufficient evidence on the best technique for peri-implant soft tissue augmentation[13].

Nevertheless, autologous connective tissue harvested from the palate and used in conjunction with a coronally repositioned flap has been successfully applied in periodontal surgery for the treatment of gingival recession, and is associated with major gains in keratinised tissue[14]. Similarly, the flap can be elevated and grafted with autologous connective tissue during the uncovering stage of two-step implant treatments. In fact, in a split-mouth study, Wiesner et al. showed that implant sites grafted with autologous connective tissue not only developed a thicker keratinised mucosa, but also achieved significantly better aesthetic scores[15]. However, despite the good clinical outcomes, the use of gingival tissue harvested from the palate comes with several disadvantages, which include the need for a second surgical site, post-operative pain, limited graft availability and more time needed for the intervention[12,16].

In order to overcome these limitations, alternative graft materials have been introduced. In particular, porcine-derived collagen has shown promising results in the treatment of gingival recession, both in terms of root coverage and keratinised tissue gain[17]. Furthermore, Cairo et al. observed, in a randomised controlled trial, that both xenogeneic dermal matrix and connective tissue grafts resulted in similar final apico-coronal keratinised tissue amounts[14].

More recently, a new xenogeneic dermal matrix has been tested in animal models as a sub-epithelial graft for the augmentation of keratinised tissue. It showed good biocompatibility and stability in the host tissue, and the same gingival thickness gain as that achieved via autologous grafting[18]. However, there have not yet been long-term clinical trials assessing its efficacy in augmenting keratinised mucosa around implant-supported restorations.

Hence, the aim of this multicentre randomised controlled trial was to evaluate the efficacy of

xenogeneic dermal matrix *versus* autologous connective graft and control treatment (no graft) in augmenting/improving the width of peri-implant keratinised mucosa and facial soft tissue levels.

The null hypothesis was that there would be no differences between groups. Secondary objectives were aesthetic outcomes (pink aesthetic score) and patient satisfaction with aesthetics.

MATERIALS AND METHODS

Study population and design

This study was set up as a randomised controlled trial of parallel group design with 3 arms. Patients were recruited from January 2015 to January 2016 in 4 universities/dental practices using similar and standardised procedures (Centre A: University of Pisa, Versilia Hospital, Pisa, Italy; Centre B: University of Brescia, Brescia Italy; Centre C: Dr. Maria Gabriella Grusovin, Gorizia, Italy; Centre D: Dr. Rossi, Genova, Italy). The plan was to recruit 24 patients per centre (8 for each group).

All patients with a single, submerged, bone level, cylindrical implant (Premium, Sweden & Martina, Due Carrare, Italy) fitted at least 3 months prior and a paucity of buccal soft tissue volume who were able to comprehend and sign an informed consent form were consecutively enrolled in the study.

Exclusion criteria were:

- History of systemic diseases that would contraindicate oral surgery;
- Long-term non-steroidal anti-inflammatory drug therapy;
- Any oral pathology involving the oral mucosa;
- Any periodontal disease affecting any residual dentition;
- Treatment with antiresorptive drugs;
- History of radiotherapy to the head/neck area;
- Unwillingness to return for follow-up examination.

All patients received thorough explanation of the study and had to complete a written informed consent form prior to being enrolled in the study. The results of the trial are reported in line with the CONSORT statement for improving the quality of reports on parallel-group randomised trials (http://www.consort-statement.org/).

The patients enrolled in the trial were treated by a single clinician at their respective centres (Centre A: Prof. Barone; Centre B: Prof. Mensi; Centre C: Dr. Grusovin; Centre D: Dr. Rossi). These discussed the inclusion and exclusion criteria, surgical procedures, outcome measures and how to measure the clinical parameters until consensus was reached at two meetings held at Versilia Hospital. All patients included in the trial were carefully assessed by examining diagnostic casts and periapical/panoramic radiographs, and data on age, gender, smoking habits and location of implant (maxillae/mandible, anterior/posterior) were collected for each patient. All patients had at least one oral hygiene session prior to abutment connection procedures in order to provide an oral environment more conducive to wound healing.

Randomisation

Using a computerised random allocation process, single implants (i.e. patients) were assigned to one of three groups: X (xenogeneic dermal matrix, Derma®), A (autologous connective tissue graft) or C (control group, no graft). An open randomisation list was sent to each centre, and the clinician in charge of surgical treatment had to assign the sequential randomisation code to consecutively enrolled patients. Random allocation was not concealed.

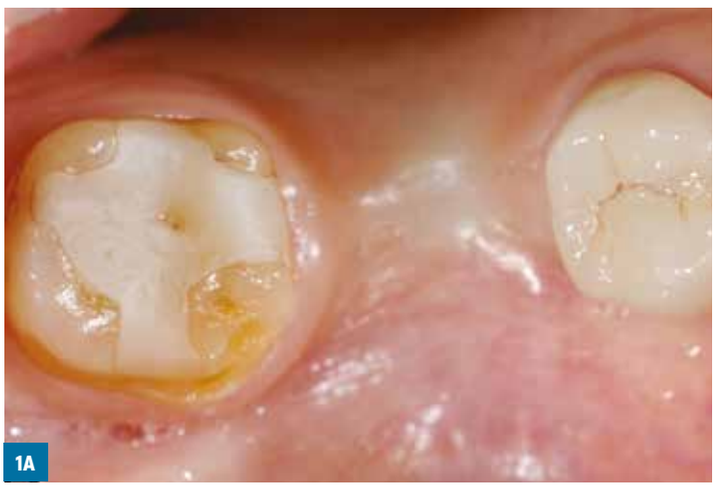
Surgical procedures

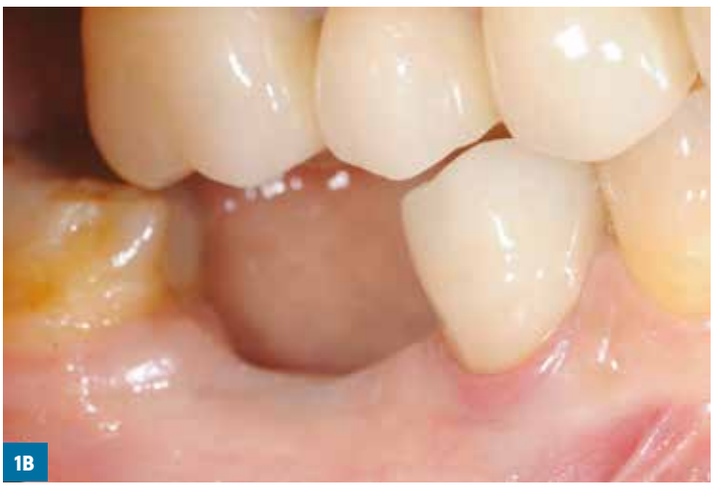
An x-ray and silicon impression were taken before surgery. All patients received prophylactic antibiotic therapy (2 g amoxicillin, or 600 mg clindamycin if allergic to penicillin) one hour before the soft tissue augmentation procedure. All patients rinsed for one minute with 0.2% chlorhexidine mouthwash immediately prior to surgery (and twice a day for the following 3 weeks with 0.12% chlorhexidine) and were treated under local anaesthesia using articaine with epinephrine 1:80,000. All surgeries were performed using the same technique by the expert clinicians, as established in the planning meetings (**FIGS. 1 A, B**).

The recipient site was prepared with a crestal incision to allow access to the implant's surgical screw. Subsequently, the experimental site was prepared by creating a split-thickness flap on the buccal side. The partial-thickness flap was separated by sharp dissection in order to prepare a periosteal bed and to eliminate muscle insertion, if present.

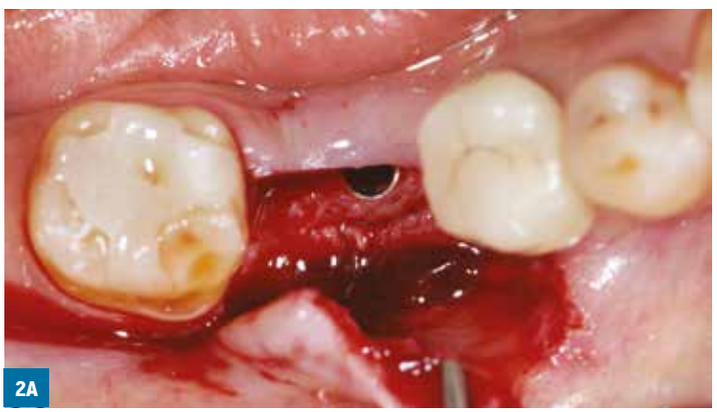
Patients enrolled in the study were assigned to the following treatment arms, according to the randomisation list provided to each single centre:

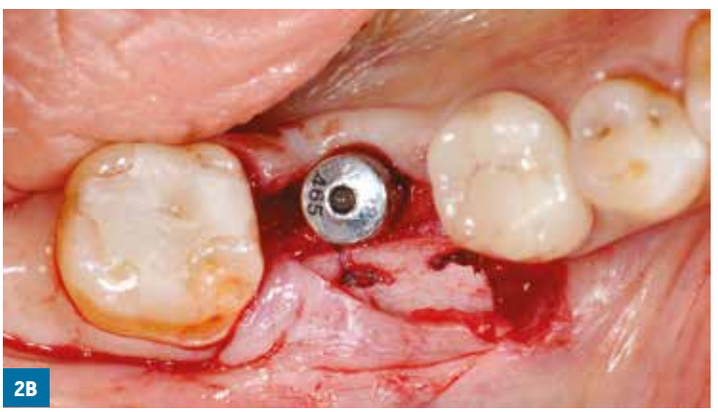
a) X: xenogeneic dermal matrix (Derma, Osteobiol, Tecnoss, Coazze, Torino, Italy), 2-mm thickness, shaped to adapt to the implant site (**FIGS. 2 A, B**);





FIGS. 1 A, B: *Preoperative image, before implant uncovering stage, showing edentulous ridge with mandibular right first molar missing and reduced keratinised tissue in xenogeneic dermal matrix group (X).*





FIGS. 2 A, B: *The recipient site was prepared with a crestal incision and split-thickness flap on the buccal side. Healing abutment positioned and xenogeneic dermal matrix placed on the buccal aspect of the implant, held in place with resorbable sling sutures in xenogeneic dermal matrix group (X).*

b) A: autologous sub-epithelial connective tissue graft, of a size established according to the receiving site and thickness of about 2 mm, harvested from the palate;
c) C (control): the soft tissues were displaced buccally and stabilised with the healing abutment but no graft.

The autologous and the xenograft connective grafts were adapted to the receiving sites and stabilised to the periosteum with a horizontal suture. The healing abutment was placed, and soft tissues were sutured to the lingual/palatal flap with 5-0/4-0 resorbable sutures.

Post-surgical instructions and prosthetic treatments

Patients were instructed to continue with antibiotic therapy, amoxicillin and clavulanic acid 1 g twice a day for 5 days, and naproxen sodium 550 mg tablets were prescribed as an anti-inflammatory, to be taken 2 times a day as long as required. Patients were instructed to avoid brushing the treated area and to use mouthwash (0.12% chlorhexidine). Any removable prosthesis was not to be worn until it had been adjusted and refitted (no sooner than 3 weeks after surgery). Sutures were removed 7-10 days after surgery, and teeth were professionally cleaned using a mild prophylaxis paste.
After 6 weeks of healing, the restorative procedures were performed: implants were manually tested for stability, impressions were taken using polyvinyl siloxane impression material and customised resin impression trays. Final prosthetic restorations were screw-retained or cemented, peri-apical x-rays and impressions were taken, and patients were enrolled in an oral hygiene programme with recall visit every 3 months (**FIGS. 3, 4**).

FIG. 3: *Soft tissue healing at 6-month follow-up in xenogeneic dermal matrix group (X).*

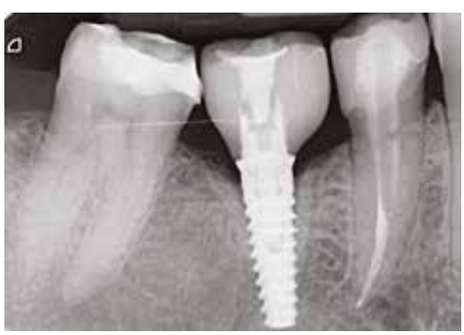


FIG. 4: *X-ray at 6-month follow-up in xenogeneic dermal matrix group (X).*

Outcome measures

One independent calibrated and blinded outcome assessor at each centre made all measurements. The following clinical parameters were recorded for all participants at baseline (T0, immediately before surgical procedures), T1 (6 weeks after soft tissue augmentation) and T2 (6 months after soft tissue augmentation).
The primary endpoint was achievement of adequate peri-implant mucosa. This was evaluated using the following parameters.

- Keratinised tissue width (KMW): measured in millimetres mid-facially from the top of the edentulous crest to the mucogingival junction, before the second stage surgery (T0, baseline), at the time of implant impression (T1) and from the gingival margin to the mucogingival junction at the delivery of the final restoration (T2).
- Facial soft tissue level (FST): evaluated measuring the distance in millimetres between the mid-facial soft tissue level and a reference line connecting the facial soft tissue level of the adjacent teeth (negative values for apical measures, positive values for coronal measures). The measurements were taken before the soft tissue augmentation (T0, baseline), and 6 weeks (T1) and 6 months after the soft tissue augmentation (T2).

The secondary endpoints were implant survival, biological complications, radiographic and aesthetic outcomes, assessed as follows.

- Prosthesis and implant failures: implant mobility, removal of stable implants due to progressive marginal bone loss or infection, and implant fracture or any other mechanical complications rendering the implant unusable. Prosthesis failure was considered as prosthesis loss secondary to implant failure or a prosthesis that had to be remade.
- Biological complications: healing and biological complications at the implant sites throughout the course of follow-up.

- Interdental papilla (IP): evaluated according to the index proposed by Jemt[19]: 0 = no papilla; 1 = less than one half of papilla height is present; 2 = greater than half of the papilla height is present, but not to the full extent of the contact point; 3 = papilla fills the entire proximal space and is in good harmony; 4 = papilla is hyperplastic. Measurements were taken at final restoration fitting (T2).
- Marginal bone level (MBL): evaluated on intra-oral radiographs at the mesial and distal sites (mMBL and dMBL) as the distance between most apical point of the marginal bone level and a reference point at the implant-abutment interface. Digital intra-oral periapical radiographs (70 KVp, 7 mA) were taken with digital sensor using a cone paralleling technique just before the second stage of surgery (T0), 6 weeks (T1) after soft tissue augmentation, and at final crown fitting (T2). A paralleling device and individualised bite blocks made of polyvinyl siloxane impression material (Flexitime, Heraeus/Kulzer, Hanau, Germany) were used to standardise the x-ray geometry.
- Aesthetic evaluation by the operator: assessments were performed using the pink esthetic score[20] at crown fitting (T2). The PES is based on seven variables: mesial papilla, distal papilla, soft-tissue level, soft-tissue contour, alveolar process deficiency, and soft-tissue colour and texture. Each variable was assessed on a 2-1-0 scale, with 2 being optimal and 0 being poor. Papilla values were evaluated for completeness, incompleteness or absence. Other variables were assessed by comparison with a reference tooth. The highest possible score reflecting a perfect match of the peri-implant soft tissue with that of the reference tooth was 14.
- Patient's aesthetic assessment: at the final restoration fitting (T2), patients provided a subjective assessment of the entire implant treatment by filling in a questionnaire, after thorough information and instruction. Patients expressed their degree of satisfaction regarding the entire implant treatment (Question 1: "What is your level of satisfaction regarding the implant treatment overall?"); the appearance of the peri-implant soft tissues (Question 2: "What is your level of satisfaction regarding the appearance of the soft tissue around your implant?"); and the appearance of the implant crown (Question 3: "What is your level of satisfaction regarding the appearance of the crown on your implant?") on a 10-cm visual analogue scale (VAS) on which 0 indicated extreme dissatisfaction and 10 complete satisfaction. Respondents' VAS scores were measured to the nearest mm on a ruler[21].

Statistical analyses

The sample size, a two-sided significance level of 0.05, a power of 90% and an expected standard deviation of 0.9 mm were established based on a previous study by Sanz et al. 2009 considering the increase in width of the KM as the main outcome variable[22]. The analysis was performed post-hoc due to the preliminary nature of these results, which are part of a larger study with a longer follow-up. The effect size (d) considered was 2 mm, and correction for multi-arm trials was applied. On the basis of these data, the minimum number of patients required to be enrolled was calculated as 24. However, considering the possibility of a certain amount of dropout (40%), the total minimum number of patients was raised to 36.
Descriptive statistics are expressed as means ± standard deviation for quantitative variables, and frequencies and percentage (%) for qualitative variables. Multiple linear regression models were created to estimate differences between groups and the centre-related effect. Fixed effects included the "Centre" and the "Group", while baseline estimates of the dependent variables were used as covariate when more than one time point was present. For marginal bone level differences, mesial and distal measurements were averaged and the resulting figure used for the inferential analysis.

Tukey's HSD was used to test all differences between least-squares experimental group means. An ordered differences report was also created. All tests were conducted at the 0.05 level of significance. Between-group differences in implant failures (dichotomous outcomes) were compared using the chi-squared test or Fisher's exact test (or the Freeman–Halton extension of Fisher's exact test) depending on the count per cell (small cell sizes with values less than 5).

RESULTS

Two centres (out of four) did not provide data for the 6-month evaluation, and this 6-month report was therefore based on reports from two centres only. Thirty-nine patients were considered eligible for the study; three patients were not enrolled due to the following reasons: 1 patient reported potentially being pregnant; 1 patient was unable to attend the planned follow-up schedule; and 1 patient had uncontrolled diabetes. Hence, 36 patients were enrolled (18 for each centre): 12 in the control group (no graft), 12 in the X group (xenograft), and 12 in the A group (autologous connective tissue graft). Three patients from the A group dropped out from the study and did not attend the final follow-up exam.

The mean age of the sample population was 50 years, and there were 20 females and 13 males; groups were balanced in terms of patient characteristics and location of graft sites (**TABLE 1**). However, there were differences between groups in terms of baseline width of keratinised mucosa and facial soft tissue level, which were 1.84 ±1.0 and 0.92 (1.4), 2.0±1.2 and 0.07 (0.8) (X), and 3.87±1.1 and 0.8 (1.1) (A) in millimetres, respectively (**FIG. 5**).

TABLE 1: PATIENTS AND SITE CHARACTERISTICS AT BASELINE (T0) BY GROUP

		Control (n = 12)	X (n = 12)	A (n = 12)
Patients	Age: mean (SD)	53.9 (7.8)	51.1 (8.2)	47.5 (5.2)
	Gender: female/male	7/5	9/3	5/7
	Smoker/ non-smoker	5/7	5/7	5/7
Sites	Maxilla/mandible	3/9	6/6	9/3
	Anterior/posterior	4/8	5/7	4/8

Control = no graft group; X = xenogeneic dermal matrix group; A = autologous connective tissue graft group.

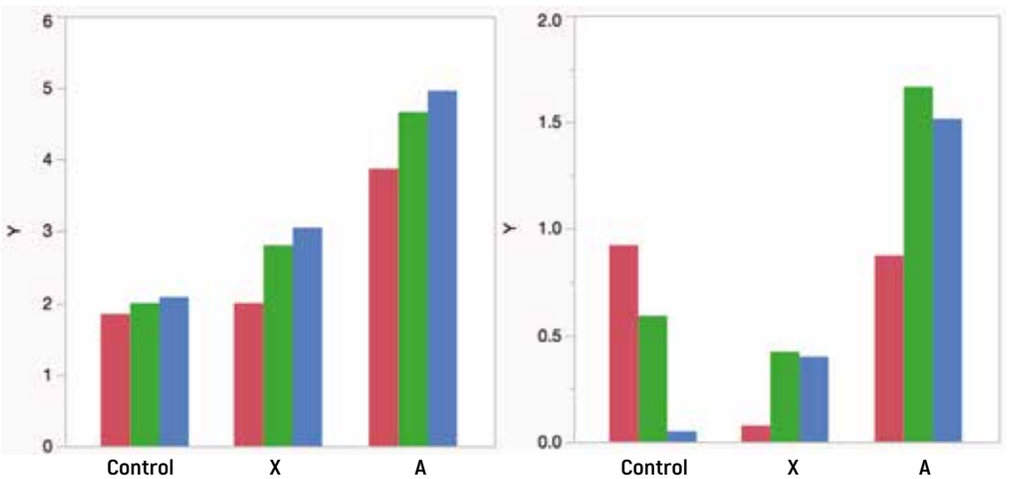


FIG. 5: *Descriptive analysis of mean width in mm of keratinised mucosa, KMW (on the left), and facial soft tissue level, FST (on the right), for the three groups at baseline (T0, red); 6-week follow-up (T1, green); and 6-month follow-up (T2, blue). Control = no graft group; X = xenogeneic graft; A, autologous connective tissue graft.*

Two implants failed (A group), respectively at 2 and 12 weeks after the second stage of surgery. The survival rate was 78% in Group A, while it was 100% in both the X and C groups (P-value 0.0682). No complications were recorded.

The primary outcomes at the three different time points (T0, T1 and T2) are reported in **TABLE 2**. At baseline, the mean keratinised mucosa width (KMW) was 1.84 ± 1.0, 2.0 ± 1.2 and 3.87 ± 1.1 mm in Groups C, X and A, respectively, i.e., the A group showed a trend toward greater initial KMW than the other groups, despite the randomised allocation of participants. At T0 the mean facial soft tissue level (FST) was 0.92 ± 1.4, 0.07 ± 0.8 and 0.8 ± 1.1 mm for Groups C, X and A, respectively.

At T2, on the other hand, the mean keratinised tissue width (KMW) was 2.08 ± 1.2, 3.05 ± 1.34 and 4.9 ± 0.8 mm, and facial soft tissue level (FST) 0.05 ± 0.9, 0.40 ± 0.47 and 1.5 ± 1.8 mm, respectively, in Groups C, X and A. Descriptive analyses conducted at T0, T1 and T2 are reported in **TABLE 2**.

The secondary outcome measures at T2 are reported in **TABLE 3**; the interdental papilla index (IP) of the mesial and distal papillae was 1.33 ± 1.0, 1.46 ± 0.9, 2.3 ± 0.5 and 1.08 ± 1.1, 1.46 ± 0.7, 2.1 ± 0.4, respectively, for Groups C, X and A.

The changes between T0, T1 and T2 in the primary outcome variable (KMW and FSL) are shown for all participants in **TABLE 4**. From T0-T2, the mean KMW increased by 0.16 ± 1.01 (P = 0.79), 1.05 ± 0.76 (P = 0.01), and 0.80 ± 1.73mm (P = 0.28) in Groups C, X and A, respectively. In xenograft group, the increase was statistically significant between both T0-T1 and T0-T2. Similarly, the mean facial soft tissue level change between T0-T2 was -0.95 ± 0.85 (P = 0.04), 0.32 ± 0.57 mm (P = 0.15) and 0.35 ± 0.79 (P = 0.30) in Groups C, X and A; therefore, A reached the highest value, but the only significant increase, of 0.34 mm (P = 0.02), was registered in the xenograft group, specifically from T1-T0.

The between-group analysis of primary outcomes for all participants is presented in **TABLE 5**. From T0 to T1 and T0-T2, the mean differences in KMW between Groups A and the control group were 2.01 ± 0.61 (P = 0.007) and 2.43 ± 0.75 mm (P = 0.009), respectively, while between Groups A and X they were 1.15 ± 0.59 (P = 0.145) and 1.37 ± 0.72 (P = 0.158). The mean differences in FST levels between Groups A and C were significant for T0-T1 and T0-T2, being 0.93 ± 0.76 (P = 0.01) and 1.32 ± 1.03 mm (P = 0.008), respectively. Other differences between groups were not statistically significant.

TABLE 2 DESCRIPTIVE ANALYSIS OF PRIMARY OUTCOMES

Outcome	Control			Xenograft			Connective tissue graft		
	N	Mean (SD)	CI 95%	N	Mean (SD)	CI 95%	N	Mean (SD)	CI 95%
KMW T0	12	1.84 (1.0)	[1.2; 2.4]	12	2.00 (1.2)	[1.2; 2.7]	12	3.87 (1.1)	[2.9; 4.8]
KMW T1	12	2.00 (1.0)	[1.3; 2.6]	12	2.80 (1.2)	[2.0; 3.5]	7	4.66 (0.5)	[4.1; 5.2]
KMW T2	12	2.08 (1.2)	[1.2; 2.8]	12	3.05 (1.3)	[2.2; 3.8]	7	4.91 (0.8)	[4.0; 5.8]
FST T0	12	0.92 (1.4)	[0.0; 1.8]	12	0.07 (0.8)	[-0.4; 0.5]	12	0.80 (1.1)	[-0.6; 1.8]
FST T1	12	0.59 (1.3)	[-0.2; 1.4]	12	0.42 (0.7)	[0.0; 0.8]	7	1.61 (1.5)	[0.0; 3.2]
FST T2	12	0.05 (0.9)	[-0.5; 0.6]	12	0.40 (0.4)	[0.1; 0.6]	7	1.51 (1.8)	[-0.4; 3.4]

N = number of patients; SD = standard deviation; and CI 95% = 95% upper and lower limits of confidence interval; KMW = width of keratinised mucosa at T0 (baseline), T1 (6-week follow-up) and T2 (6-month follow-up); FST = facial soft tissue level at T0, T1 and T2.

TABLE 3 DESCRIPTIVE ANALYSIS OF SECONDARY OUTCOMES AT T2 (6 MONTHS AFTER GRAFT SURGERY)

Outcome	Control			Xenograft			Connective tissue graft		
	N	Mean (SD)	CI 95%	N	Mean (SD)	CI 95%	N	Mean (SD)	CI 95%
mIP	12	1.33 (1.0)	[0.6; 2.0]	12	1.46 (0.9)	[0.8; 2.0]	7	2.30 (0.5)	[1.7; 2.8]
dIP	12	1.08 (1.1)	[0.3; 1.8]	12	1.46 (0.7)	[0.9; 1.9]	7	2.10 (0.4)	[1.7; 2.5]
Question 1	12	92.08 (8.3)	[86.7; 97.4]	12	92.53 (8.6)	[87.2; 97.2]	7	97.51 (4.1)	[93.1; 101]
Question 2	12	92.08 (8.3)	[86.7; 97.4]	12	92.91 (7.0)	[88.6; 97.1]	7	94.10 (4.9)	[89; 99.3]
Question 3	12	92.50 (8.6)	[86.9; 98.0]	12	96.01 (7.6)	[91.4; 100]	7	100 (0)	[100; 100]
PES: mPapilla	12	1.41 (0.6)	[1.0; 1.8]	12	1.08 (0.5)	[0.8; 1.4]	7	1.57 (0.5)	[1.1; 2.1]
PES: dPapilla	12	1.25 (0.7)	[0.8; 1.7]	12	0.92 (0.5)	[0.6; 1.2]	7	1.57 (0.5)	[1.1; 2.1]
PES: Curvature F.	12	1.50 (0.9)	[0.9; 2.1]	12	1.42 (0.5)	[1.1; 1.7]	7	1.71 (0.5)	[1.3; 2.2]
PES: Level P.	12	1.42 (0.7)	[1.0; 1.8]	12	1.50 (0.5)	[1.2; 1.8]	7	1.57 (0.5)	[1.1; 2.1]
PES: Alveolar P.	12	1.00 (0.7)	[0.5; 1.5]	12	1.33 (0.5)	[1; 1.6]	7	1.43 (0.5)	[0.9; 1.9]
PES: Soft tissue C.	12	1.50 (0.5)	[1.2; 1.8]	12	1.92 (0.3)	[1.7; 2.1]	7	2.01 (0)	[2.0; 2.0]
PES: Soft tissue T.	12	1.58 (0.5)	[1.3; 1.9]	12	1.67 (0.5)	[1.4; 2.0]	7	1.71 (0.5)	[1.3; 2.2]
Tot. PES (max 14)	12	9.67 (3.3)	[7.6; 11.8]	12	9.83 (1.5)	[8.9; 10.8]	7	11.57 (1.5)	[10.2; 13.0]
mMBL	12	1.83 (0.8)	[1.3; 2.3]	12	1.29 (0.7)	[0.8; 1.7]	7	0.75 (0.8)	[-0.1; 1.6]
dMBL	12	1.66 (0.8)	[1.1; 2.2]	12	1.22 (0.7)	[0.7; 1.6]	7	0.96 (0.7)	[0.1; 1.7]
Tot MBL	12	1.74 (0.8)	[1.1; 2.3]	12	1.25 (0.7)	[0.7;1.7]	7	0.85 (0.8)	[-0.1; 1.7]

N = number of patients; SD = standard deviation; CI 95% = 95% upper and lower limits of confidence interval; mIP and dIP = mesial and distal interdental papilla indices; Questions 1–3 = patient's aesthetic assessment; PES = pink aesthetic scores for mesial papilla, distal papilla, curvature of the facial mucosa, level of the facial peri-implant mucosa, alveolar process deficiency, soft tissue colour, soft tissue texture at the facial aspect of the implant site; mMBL and dMBL = mesial and distal marginal bone levels.

The between-group analyses of secondary outcomes are shown in **TABLE 6**, radiographic measures, and **TABLE 7**, aesthetic outcomes. As regards the aesthetics evaluation by the operator, at T2 there were no significant differences in pink esthetic score (PES) between the three groups: between A and C the difference was 1.93 ± 2.45 (P = 0.14), between A and X it was 1.16 ± 2.50 (P = 0.49), and between X and C it was 0.77 ± 2.16 (P = 0.65). The only significant difference in interdental papilla index was between Groups A and C at T2, when it was 1.07 and 1.15 respectively for mIP and dIP (P = 0.04; P = 0.01).

As far as patients' mean aesthetic satisfaction is concerned, this was high at T2 for all three groups, at 92.2 ± 8.4 (C), 93.8 ± 7.7 (X) and 97.2 ± 3.0 (A). There were no statistically significant differences between the three groups in terms of aesthetic outcomes.

For each between-groups analysis, the influence of the centre (Clinic A and Clinic B) was also calculated: for primary outcomes only the T0-T2 FST was significant, at 0.67 ± 0.62 mm (P = 0.0348). Considering the secondary outcomes, the difference in PES was statistically different, at 2.81 ± 1.33 (P = 0.0002) (Centre A = 5.61; Centre B = 8.42), while the mean difference in patient satisfaction with aesthetics, 9.10 ± 4.88 (P = 0.0007), was in favour of Centre B: for Question 1 (Centre A = 89.5; Centre B = 98.69); Question 2 4.84 ± 5.24 P = 0.06 (Centre A = 90.6; Centre B = 95.5); and Question 3 6.73 ± 4.87 P = 0.0095 (Centre A = 92.8; Centre B = 99.6), respectively.

TABLE 4 WITHIN-GROUP CHANGES IN PRIMARY CLINICAL OUTCOMES

Outcome	Group	Time	Mean difference	Standard error	95% CI	P-value
DFST	Control	T1–T0	-0.40	0.23	[-0.91; 0.09]	0.1041
		T2–T0	-0.95	0.42	[-1.8; -0.02]	0.0455*
		T2–T1	-0.54	0.32	[-1.25; 0.16]	0.1212
	X	T1–T0	0.34	0.13	[0.05; 0.63]	0.0218*
		T2–T0	0.32	0.21	[-0.13; 0.78]	0.1527
		T2–T1	-0.02	0.19	[-0.45; 0.40]	0.9089
	A	T1–T0	0.50	0.22	[-0.07; 1.07]	0.0756
		T2–T0	0.35	0.30	[-0.44; 1.14]	0.3095
		T2–T1	-0.15	0.27	[-0.85; 0.55]	0.6094
DKMW	Control	T1–T0	0.08	0.31	[-0.60; 0.77]	0.7949
		T2–T0	0.16	0.45	[-0.84; 1.17]	0.7227
		T2–T1	0.08	0.33	[-0.65; 0.82]	0.8088
	X	T1–T0	0.80	0.36	[0.01; 1.60]	0.0470*
		T2–T0	1.05	0.35	[0.28; 1.81]	0.0111*
		T2–T1	0.24	0.17	[-0.14; 0.63]	0.1923
	A	T1–T0	0.50	0.56	[-0.94; 1.94]	0.4150
		T2–T0	0.80	0.67	[-0.93; 2.53]	0.2881
		T2–T1	0.30	0.19	[-0.19; 0.79]	0.1780

FST = facial soft tissue level; KMW = keratinised tissue width between different time points (T0, baseline; T1, 6-weeks follow-up; T2, 6-month follow-up); Control = no graft group; X = xenogeneic dermal matrix group; A = autologous connective tissue graft group.

Mean differences, standard error, and as CI 95%, 95% upper and lower limits of confidence interval. Statistically significant p-values are marked with an asterisk(*).

TABLE 5 BETWEEN-GROUP DIFFERENCES IN PRIMARY CLINICAL OUTCOMES

Time	Outcome	Level	-Level	Mean Difference	Standard error	95% CI	P-value
DT1–T0	FST	A	Control	0.93	0.30	[0.17; 1.69]	0.0142*
		X	Control	0.57	0.26	[-0.07; 1.22]	0.0914
		A	X	0.35	0.31	[-0.43; 1.15]	0.5084
	KMW	A	Control	2.01	0.61	[0.49; 3.54]	0.0078*
		A	X	1.15	0.59	[-0.31; 2.61]	0.1455
		X	Control	0.86	0.39	[-0.12; 1.86]	0.0956
DT2–T0	FST	A	Control	1.32	0.41	[0.30; 2.35]	0.0089*
		X	Control	0.66	0.35	[-0.20; 1.53]	0.1600
		A	X	0.66	0.42	[-0.39; 1.72]	0.2832
	KMW	A	Control	2.43	0.75	[0.56; 4.30]	0.0090*
		A	X	1.37	0.72	[-0.42; 3.17]	0.1583
		X	Control	1.05	0.49	[-0.16; 2.27]	0.0995

FST = facial soft tissue level; KMW = keratinised tissue width between different time points (T0, baseline; T1, 6-weeks follow-up; T2, 6-month follow-up); Control = no graft group; X = xenogeneic dermal matrix group; A = autologous connective tissue graft group.

Mean differences, standard error, and as CI 95%, 95% upper and lower limits of confidence interval. Statistically significant p-values are marked with an asterisk(*).

TABLE 6 BETWEEN-GROUP DIFFERENCES IN SECONDARY RADIOGRAPHIC OUTCOMES

Time	Outcome	Level	-Level	Mean Difference	Standard error	95% CI	P-value
DT2–T0	MBL	X	A	0.40	0.15	[0.01; 0.79]	0.0244*
		Control	A	0.47	0.16	[0.05; 0.89]	0.0430*
		X	Control	0.07	0.13	[-0.25; 0.40]	0.8485

MBL = mean of mesial and distal marginal bone loss between groups at different time points T0, baseline; T1, 6-weeks follow-up; T2, 6-month follow-up); Control = no graft group; X = xenogeneic dermal matrix group; A = autologous connective tissue graft group.

Mean differences, standard error, and as CI 95%, 95% upper and lower limits of confidence interval. Statistically significant p-values are marked with an asterisk(*).

TABLE 7 BETWEEN-GROUP DIFFERENCES IN SECONDARY AESTHETIC OUTCOMES AT T2 (6 MONTHS AFTER GRAFT SURGERY)

Outcome	Level	-Level	Mean Difference	Standard error	95% CI	P-value
PES	A	Control	1.93	0.99	[-0.52; 4.38]	0.1436
	A	X	1.16	1.01	[-1.33; 3.66]	0.4914
	X	Control	0.77	0.87	[-1.39; 2.93]	0.6527
mIP	A	Control	1.07	0.42	[0.00; 2.13]	0.0485*
	A	X	0.77	0.42	[-0.27; 1.82]	0.1813
	X	Control	0.29	0.34	[-0.56; 1.16]	0.6750
dIP	A	Control	1.15	0.39	[0.16; 2.14]	0.0195*
	A	X	0.60	0.39	[-0.37; 1.58]	0.2939
	X	Control	0.55	0.32	[-0.25; 1.35]	0.2211
QA	A	Control	6.17	3.25	[-1.90; 14.25]	0.1595
	A	X	3.91	3.22	[-4.07; 11.90]	0.4555
	X	Control	2.26	2.64	[-4.29; 8.88]	0.6723
QB	A	Control	2.48	3.50	[-6.19; 11.16]	0.7595
	X	Control	1.80	2.84	[-5.24; 8.83]	0.8029
	A	X	0.68	3.46	[-7.89; 9.2]	0.9787
QC	A	Control	8.06	3.30	[-0.14; 16.26]	0.0549
	X	Control	4.83	2.68	[-1.82; 11.50]	0.1886
	A	X	3.22	3.27	[-4.89; 11.33]	0.5927

PES = pink aesthetic score; mIP and dIP = mesial and distal interdental papilla indices, respectivey; Q1–Q3 = questions 1–3 on patients' aesthetic satisfaction questionnaire; Control = no graft group; X = xenogeneic dermal matrix group; A = autologous connective tissue graft group.

Mean differences, standard error, and as CI 95%, 95% upper and lower limits of confidence interval. Statistically significant p-values are marked with an asterisk(*).

DISCUSSION

The role of keratinised mucosa in maintaining soft tissue health was expressed early, in 1972, by Lang & Loe[9]. More recently, articles have correlated the presence of keratinised tissue with aesthetic value and soft tissue stability around dental implants[23], even though Todisco et al. reported that the height of the keratinised mucosa does not seem to alter clinical outcomes

around implant sites[6]. That being said, In 2008, a systematic review concluded that although the presence of peri-implant keratinised mucosa does not seem to alter long-term implant survival, it can influence the biological outcome[8]. Recently, for example, Gobbato et al. highlighted the benefits of keratinised tissue in the prevention of plaque accumulation and inflammation of peri-implant mucosa[24]. This has been confirmed by some studies showing that having less than 2 mm of keratinised tissue is a risk for bleeding on probing, and is more likely to cause discomfort during home oral hygiene[10,11].

Hence, our trial was designed to focus on determining the best strategy for improving the width of keratinised peri-implant soft tissues at second-stage implant surgery, whether autologous sub-epithelial connective tissue graft or xenogeneic dermal matrix, comparing with no-graft controls.

Our preliminary results revealed no differences between the xenograft and autologous tissue grafting in the width of keratinised mucosa around the implant, but that both yielded significant increases (1.05 and 0.80 mm), respectively from baseline to six-month follow-up. On the whole, these results are similar to those obtained in a previous RCT on sixty implants by Cairo et al. 2017[25], who reported increases in keratinised mucosa width of 1.1 and 0.9 mm for xenogeneic collagen matrix and connective tissue graft groups, respectively.

Similarly, both test procedures in our RCT achieved similar facial soft tissue level increases (vertical), with no significant differences between groups. In particular, the autologous graft resulted in a mean 0.35 mm increase and the xenograft 0.32 mm after 6 months, in comparison to the no-graft controls, which lost 0.95 mm after implant uncovering. This contrasts somewhat with findings from an RCT by Froum et al. 2015[26], who also found no statistical differences between peri-implant vertical soft tissue height, which, however, increased by 0.78 mm after xenogeneic matrix graft and 0.14 mm after no graft.

The importance of vertical soft tissue has been analysed by Puisys & Linkevicius[27], who suggested that a good quantity of vertical soft tissue may be significant in preventing bone resorption around implants. This has been contrasted by findings by other authors, who reported that keratinised mucosa at vestibular and lingual sites is associated with an increased marginal bone loss as compared to implants having at least one side without it[6]. Our RCT found no between-group differences in marginal bone loss, but this could be related to the short follow-up of six months covered by this preliminary report.

Furthermore, we detected no significant difference between groups at T2 in terms of either the Jemt papilla index[28] or the pink esthetic score[20] methods of papillae analysis. However, the autologous graft group showed better aesthetic outcomes, albeit not statistically so, from both the operator and patients' perspectives, with VAS analysis by the latter confirming that soft tissue augmentation is related to good patient satisfaction[28].

That being said, the aesthetic outcomes and overall satisfaction were very high in all three groups. Indeed, there were no significant differences between groups in this regard or in terms of soft tissue complications at six-month follow-up. This contrasts with findings from a split-mouth study by Wiesner et al., which reported that implant sites grafted with autologous connective tissue achieve significantly better aesthetic scores than the no grafted control group[15].

Like in our study, however, other authors have reported patients being highly satisfied with final aesthetic outcomes across the board, with no significant difference between xenograft and autologous graft groups[24]. Nonetheless, it should be considered that, despite comparable clinical outcomes, autologous grafting involves harvesting tissues from the palate, and comes with the associated disadvantages, such as the need for a second surgical site, post-operative pain[18], and more time required for surgical procedures[12,24,29].

As regards limitations, this study was based on a small sample size and a short, albeit preliminary, follow-up period. However, we plan to conduct longer-term patient follow-up over a period of three years. Other limitations were the absence of allocation concealment and a lack of calibration of the assessors from each centre. Moreover, two out of four centres did not provide any data for the 6-month analysis.

CONCLUSIONS

The outcomes of the present study reveal that autologous connective tissue grafting can attain significant gains in facial soft tissue height and keratinised mucosa width as compared to no-graft controls at six months. Nonetheless, at this early stage, no clinical advantages of augmenting the soft tissues versus not doing so were apparent.

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