Subepithelial Connective Tissue Graft versus Amniotic Chorion Membrane for Peri-implant Mucosal Thickness Enhancement.

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In

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Introduction

Soft tissue grafting procedures are increasingly performed for a number of indications in conjunction with dental implant therapy[1]. Major clinical indications include gain of keratinized tissue (KT) and increase peri-implant mucosal thickness (PMT) which improve functional, aesthetic, and biological outcomes after therapy[2].

Various techniques and materials have been used to thicken the peri-implant mucosa. These include connective tissue grafts (CTGs)[3], platelet-rich fibrin (PRF)[4], acellular dermal matrix grafts[5] and xenogeneic collagen matrix[6].

Soft tissue augmentation using subepithelial connective tissue graft (SCTG) harvested from the hard palate or tuberosity region has become the gold standard technique to thicken peri-implant tissue and to improve aesthetic outcomes[3]. However, SCTG has been criticized to be associated with the risk of complications during and after surgery such as bleeding, infection or necrosis[7, 8]. In addition, there is limitation in quality and quantity of tissue that is available for grafting. Moreover, the harvesting procedure at the donor site may be associated with increase morbidity due to graft harvesting[8].

As a consequence, an acellular dermal matrix (ADM) allograft has been used as a SCTGs replacement. The ADM allograft is obtained from an allograft donor skin and produced by a carefully controlled process that removes the epidermis and dermis cells without altering the extracellular matrix structure which provides the basis for cellular in-growth and subsequent tissue remodelling[9]. However, these grafts are very thin due to the manufacturing process and showed an increased shrinkage rate[10].

Besides, histological analysis of tissue augmented with ADM did not resemble native oral soft tissue, but had a more scar-like appearance[11].

Recently, Amniotic Chorion membrane (ACM) has been proposed to augment keratinized tissues around teeth and dental implants. ACM is the inner most lining of the fetal membrane that is in contact with the developing fetus[12]. The ACM has numerous advantages owing to its structure and composition. The extracellular matrix comprises collagen Types I, III, IV, V, and VI and cell-adhesion bioactive factors, such as fibronectin and laminin[13].

Collagen is well tolerated and bioabsorbable, has haemostatic properties, and encourages migration of adjacent autogenous connective tissue. Fibronectin is involved in many cellular processes, including tissue repair, blood clotting, cell migration, and adhesion[14].

Laminin and Laminin-5 has a high affinity for binding epithelial cells in contrast to traditionally available membranes[15] This biological factor allows the ACM to be left exposed to the oral environment. It is believed that the antibacterial property of ACM is related to the presence of lysozyme present in amniotic fluid[16] .

According to Talmi and coworkers[17], lysozyme is a powerful bactericidal enzyme in high concentration in ACM, and it acts against many gram-negative microorganisms. Some studies have suggested that ACM is relatively well tolerated when used as a self-graft[18, 19].

It has been also proven that the ACM has a potential for regeneration in periodontology as the matrix of the chorion contains abundant growth factors, such as keratinocyte growth factor, basic fibroblast growth factor,

and transforming growth factor-β, that promote periodontal regeneration and provide a natural environment for accelerated healing[20]. It was used in socket preservation, guided tissue regeneration and guided bone regeneration. Furthermore, the ability of this allograft to self-adhere eliminates the need for suturing[15, 21].

To the best of our knowledge, there is still a scarcity of literature evaluating the feasibility of ACM. Furthermore, there have been no studies conducted to evaluate the clinical efficacy using SCTG compared to ACM simultaneously with dental implant placement.

The hypothesis states that using ACM membranes will produce equivalent results in terms of PMT enhancement around dental implants in comparison to SCTG graft in humans. The present study will be performed to compare and evaluate clinically and radiographically the soft tissue healing around dental implants augmented with subepithelial connective tissue graft versus Amnion Chorion Membrane.

Aim of the work

The objective of this study will be to assess the efficacy of the peri-implant mucosal thickness enhancement following either subepithelial connective tissue grafts or Amnion Chorion Membrane placed simultaneously with dental implant placement.

Materials and Methods

Study design:

A Randomized clinical study, single blinded, with equal randomization, active controlled, with Allocation ratio 1:1.

Study setting:

The research will be carried out in the Periodontology clinic, Faculty of Dentistry, Tanta University.

Ethical consideration:

The Purpose of the present study will be explained to the patients and informed consent will be obtained according to guidelines on human research adopted by the Research Ethics Committee (REC) at Faculty of Dentistry, Tanta University.

Sample size calculation:

The minimal sample size is calculated based on a previous study aimed to evaluate the early volumetric changes after buccal soft tissue contour augmentation around implants with a porcine collagen matrix (CM) versus the subepithelial connective tissue graft (SCTG) from the palate.[22] Schmitt et al. (2021)[22] reported that the mean soft tissue thickness increase (mm) in the buccal contour after 6 months was 0.30 ± 0.16 mm (CM) and 0.80 ± 0.61 mm (SCTG). The sample size was calculated to detect difference in increase in the soft tissue thickness. Based on Schmitt et al. (2021)[22] results, and adopting a power of 80% (β=0.20) to detect a standardized effect size in increase in the soft tissue thickness (primary outcome) of 1.121, and level of significance 5% (α error accepted =0.05), the minimum required sample size was found to be **11 patients per group** (number of groups=2) **(Total sample**

size=22 patients)[23, 24]. After adjustment for a dropout rate of 10%, the sample size was increased to **13 patients per group** (number of groups=2) **(Total sample size=26 patients)**.[25]

Software:

The sample size was calculated using G Power version 3.1.9.2 [26]. The equation is:

$$N = \frac{(r+1)(Z_{\propto/2} + Z_{1-\beta})^2 \sigma^2}{rd^2}$$

Where Z_a is the normal deviate at a level of significance and Z_{1-b} is the normal deviate at 1-b% power with b% of type II error

Sites selection:

Twenty-six sites that are indicated for peri-implant mucosal augmentation at the time of implant placement will be selected from the Periodontology Clinic, Faculty of Dentistry, Tanta University. Participants will be randomized to the control (subepithelial connective tissue graft) or test (amniotic chorion membrane) group. To include the patients in this study their written consent will be obtained, and all the procedure will be explained before the treatment.

Inclusion criteria:

- Patients with good systemic health with no contraindication for periodontal surgery.
- No history of any medications in the previous 6 months that may interfere with periodontal tissue health or healing.
- Ability to maintain good oral hygiene as evidenced in recall visits.
- Aged 30 to 55 years.

- Stable periodontal condition and missing one single tooth (maxillary anterior teeth and premolars) with adjacent teeth present and thin mucosal phenotype (< 2mm bucco- lingual thickness).

Exclusion Criteria:

- Medically compromised patients and systemic conditions precluding periodontal surgery.
- Smokers.
- Severe hematologic disorders (e.g., hemophilia or leukemia), uncontrolled infectious or metabolic diseases that could compromise normal healing, liver, or kidney dysfunction/failure.
- Patients subjected to irradiation in the head and neck area.
- Patients treated or under treatment with intravenous amino bisphosphonates.
- Patient affected by active periodontitis or has poor oral hygiene and motivation.
- Uncontrolled diabetes mellitus.
- Pregnant women or planning to become pregnant, and nursing mothers.

The clinical study design:

1. Phase I therapy

- All patients will receive a comprehensive periodontal examination, oral hygiene instructions and they will be subjected to full mouth scaling, root planning (SRP) and polishing.
- Sites with occlusal trauma will be subjected to occlusal adjustment.
- One month following phase I therapy, patients who qualify the inclusion criteria for surgical procedures in terms of oral hygiene measures and compliance will be selected.

2. Surgical Phase

- The sites will be classified randomly by the sealed envelope technique into two treatment groups, 13 sites in each group.

Group 1 (Control Group)

Autogenous SCTGs + dental implants*.

Group 2 (Test Group)

ACM**+ dental implant

Prior to the initiation of study procedures, Peri-implant Mucosal Thickness (PMT) at three different heights (1, 3, and 5 mm apical from the mucosal margin) will be measured using a custom stent and an endodontic spreader. Following enrolment, each participant will be randomly assigned using the sealed envelope technique by a masked investigator, to one of two treatment groups: Control (implant placement with autogenous SCTG harvested from the palatal mucosa); or test (implant placement with ACM). Participant allocation will be withheld from the surgeon until shortly before the surgical visit.

At the baseline surgical visit, local anaesthetic will be administered; the buccal PMT will be measured and recorded. A periodontal probe (UNC-15) and an endodontic spreader will be gently inserted through the custom stent to the surface of the mucosa and the bone, respectively, at approximately 1, 3, and 5 mm apical to the estimated free mucosal margin, using the gingival zenith of the adjacent teeth as a reference. The difference

* Biodem implant - Germany
** BioXclude, USA

between the measurement from the stent to the mucosa (using the probe) and from the stent to the bone (using the endodontic spreader) as the PMT.

Mid-buccal keratinized mucosal width (KMW) at the edentulous site using a periodontal probe (UNC-15) will be recorded, as well.

Surgical procedure

First surgery

All patients will be premedicated with antibiotic prophylaxis (Augmentin 2,000 mg or Clindamycin 600mg), depending on the history of drug allergy, 1 hour prior to implant placement surgery. Clinical photographs will be taken prior to, during, and after surgery.

All surgical procedures will be performed under strict aseptic conditions and all patients will be injected with local anaesthesia (Articaine 4% 1:100 000 epinephrine).

Paracrestal incisions extended through the papillae on either side of the implant site will be made. Vertical releasing incisions will be made, when it is necessary, on each side of the implant to facilitate the planned advancement of the flap tissue to cover the grafts. A combination of full thickness and partial thickness flap design will be employed to create a recipient bed for the graft and the implant will be placed in an ideal position according to the cone beam CT (CBCT) scan.

Following baseline measurements, the implant will be placed according to the manufacturer's protocol, along with the provided cover screw. For the control group, a connective tissue graft will be harvested from the palatal mucosa. For the test groups, the ACM will be prepared according to the manufacturer's instructions and trimmed to appropriate size.

The grafts will be positioned over the coronal and buccal aspect of the alveolar ridge and secured to the recipient sites with non-absorbable sutures anchored in the buccal periosteum and the palatal flap, and then passively covered with the primary flap. The flaps will be sutured with multiple simple interrupted and double vertical loop sutures to achieve primary closure in all cases using non-absorbable sutures.

3. Postoperative Care

- Patients will be continued to take the same antibiotics for 7 days. They will be instructed to rinse twice daily with a chlorhexidine mouth-rinse (0.12%) during the first 2 weeks and to avoid brushing and interdental cleaning next to the treated area. They also will be told to avoid chewing on, or inflict any trauma, to the treated area.
- Patients will return to the clinic 7 to 14 days post-surgery for suture removal, Final crown cementation after 6 months.

4. Outcomes variables

- **The primary outcome** of interest will be to measure changes (in mm) in horizontal PMT on the buccal aspect of the edentulous alveolar ridge from baseline (BL) (implant placement and grafting) to (3 and 6 month) of post-surgical healing.
- Prior to surgery (baseline), at 3 and 6 month, impressions of the grafted sites will be taken including at least the two neighbouring teeth and using an Addition silicone impression material. Dental stone casts will be fabricated and optically scanned with a desktop 3D scanner. Digital models of each time-point per patient will be captured as stereolithography (STL) files. Subsequently, these STL files will be imported into a digital imaging software program for analysis of the volumetric changes in the grafted areas. The images of the baseline and

follow-up datasets will be superimposed and matched using the best-fit algorithm at the adjacent tooth surfaces. After definition of specific regions of interest, the software will calculate the volumetric, changes will be measured in mm, which corresponded to the mean distance between the three surfaces representing the evaluated time-points (BL, 3 and 6 month).

- **The secondary outcome** of interest will include change (in mm) in mid-buccal (KMW) in an apico-coronal direction. Assessment of wound healing at different post-surgical time points using a modified wound healing index that was introduced by Huang et al.,(18) which evaluates periodontal soft tissue wound healing with scores from 1 to 3. On this scale, wounds are scored at baseline of surgery and at 4,8,12,24 weeks post-operative.
- The modified wound healing index (MWHI) included three categories:
  1) Uneventful wound healing with no or minimal mucosal edema or erythema, and no suppuration or graft exposure.
  2) Normal wound healing with slight to moderate mucosal edema, erythema and/or graft exposure, but no suppuration.
  3) Poor wound healing with significant mucosal edema, erythema, graft exposure, and suppuration.

- Patient-reported outcome measures (PROMs): self-reported postoperative discomfort and overall satisfaction upon study completion using a 100-point visual analog scale (VAS) recorded at 1,3,7,15 days post operatively.

Statistical analysis:

Data will be collected and analysed using Statistical Package for Social Science (SPSS) program for statistical analysis. Data validation for entry

mistakes will be carried out. The normality of distribution will be checked to determine the appropriate statistical approach to be adopted. Level of Alpha error will be set to 5% with a significance level of 95%. Statistical significance will be tested at p value <0.05.

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