Clinical Evaluation of the Use of Surgical Tube Technique versus Hyaluronic Acid Gel Injection in Reconstruction of the Interproximal Papilla in Patients with Class II Papillary Deficiency

(Randomized Clinical Trial)

التأثير الاكلينيكي للأنبوب الجراحي مقارنة بحقن حمض الهيالورونيك في اعاده بناء الحليمه البينيه في حالات العيوب اللثويه المستوي الثاني (التجارب السريرية العشوائية)

Protocol submitted to
Faculty of Dentistry, Cairo University
for partial fulfillment of the requirements for the PhD Degree in periodontology

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2020

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I. Administrative information:

1. Title

The clinical effect of surgical Tube technique versus Hyaluronic Acid gel injection in reconstruction of the interproximal papilla in patients with class II papillary defect. (Randomized clinical Trial).

2. Protocol Registration

3. Protocol version

First version.

4. Funding

Self-funding.

5. Roles and responsibilities

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II. Introduction:

A. Background and rationale:

6. Research question:

In papilla class II defect will tube technique correct the papillary loss to fulfill complete gain in papillary height and embrasure fill compared to Hyaluronic acid injection?

The full occupation of the interdental papilla at the entire embrasure space apical to the interdental contact point/area., especially anteriorly, has been one of the most important aspects in increasing public demands for esthetics. Added to the esthetically unaccepted black triangle, loss of interdental papilla results in food impaction and plaque accumulation. Thus, negatively affects the integrity of the periodontal tissue health ***(Burke etal 1994).***

Knowing that the interdental space is surrounded by avascular root surfaces on either side, the feeding source of blood supply comes from the gingivo-papillary unit and the crest of the alveolar bone. Any tear or excess trauma to this narrow band of papillary tissue results in compromised vascularity which leads to necrosis. This gives attention for the urgency of

maintaining papillary integrity during all dental procedures and lessening traumatization to consider preventing its loss ***(Zucchelli 2008).***

Several attempts for augmentation and rebuilding techniques aiming at reconstructing the defective papilla encountered major limiting factor pointing at the minor blood supply feeding the small fragile interdental papilla ***(Georgieva, etal. 2017;Alahmari 2018; Thomas etal. 2000).***

The injection of hyaluronic acid gel have been considered of the latest conventional non invasive techniques for treatment of deficient papilla to escape drawbacks of surgeries however still entails unpredicted complete gingival papilla improvement owing to the relative compact nature of the gingiva which is influenced by the gingival biotype that could hinder the positive outcomes on favor of thick biotype that could help to enrich the effect of hyaluronic acid thru accelerating the thick connective tissue related abundance of gingival fibroblast proliferation, promoting the generation of collagen fibers ***(Becker etal. 2010).***

Karring etal 1974 tested papillary tissue augmentation through influencing connective tissue cell proliferation, providing clot stability, protecting the reconstructed site during wound healing. He reported that sub-epithelial connective tissue possess the genetically determined factor to induce changes in the differentiation of the nature of the epithelium developing at a region. Sub-epithelial connective tissue graft has been successfully used for the double blood supply it provides, closer color blend of graft to the adjacent tissue and absence of keloid healing.

However, because of the low blood supply and the anatomic restriction of the recipient gingival papilla, the result is unpredictable unless successful surgical attempt aiming for clot stability influenced by the defect related flap design, to avoid early wound failure and exposure of the treated area occurred ***(Langer etal. 1985; Han & Takei 2000).***

Many surgical approaches using traditional periodontal plastic and augmentation procedures have been proposed to overcome the problem of the low blood supply. However, Due to the number and location of incisions, which can disrupt the blood supply, techniques with new flap designs were found to be invasive with limited success and long-term stability needed for complete healing ***(Carranza & Zogbi 2011; Singh etal 2013).***

A new surgical technique called Tube technique has been developed for protection of the

wound without disturbing the blood supply of the graft by precise flap design allowing containment of the graft within a rich vascular enclosed bed, thereby predictably offering reliable and satisfactory results ***(Hooshang etal 2020).***

Our randomized clinical trial target is comparing between the clinical efficacy of surgical Tube technique and Hyaluronic Acid gel injection in reconstruction of the interproximal papilla in patients with class II papillary defect.

Statement of the problem:

Restoring defective interdental papilla partially or totally in structure and function creates a challenge in dental field owing to the small working space and limited blood supply to the area.
This led to the innovation of different techniques for treatment of defective interdental papilla that aimed at papillary tissue augmentation reported to be attained through influencing connective tissue cell proliferation, providing clot stability, protecting the reconstructed site during wound healing ***(Nordland, 2018;Singh etal. 2013)***

Among these techniques is the injection of hyaluronic acid gel have been considered of the latest non-invasive technique to escape drawbacks of surgeries. Though proved to have some positive results in the literature however still entails unpredicted complete gingival papilla improvement because of the diverse injection methods, cycles, dosage, and the course of treatment that needs further optimization, in addition to the lack of unified patient inclusion criteria, especially the gingival biotype ***(Langer etal. 1985;Han & Takei 2000).***

Several surgical techniques designed to host the richest effective known sub-epithelial connective tissue graft had failed to allow maximum benefit of the graft due to the disadvantages from the number and location of incisions of their flap design, which possibly disrupt the blood supply needed added to unconsidered flap thickness, and flap retraction that impacted the success of soft tissue grafting ***(Singh etal 2013;Al-Zarea etal. 2015).***

Surgical Tube technique has recently been developed optimizing flap thickness, flap retraction and tension to escape disturbance of the blood supply of the graft by its use of apical incisions and containment of the graft within a rich vascular enclosed bed, thereby offering an attempt to guarantee integrity of soft tissue with no disruption of blood supply ***(Hooshang etal 2020).***

Rationale for conducting the research:

1. Complete Regeneration of the defective interdental papilla has been evasive in the literature. Many Attempts failed to fully restore the integrity of interdental papilla that protects the periodontal structure aiming at longstanding survival of interdental tissue facing negative destructive factors, and following current directions of dentistry to esthetic purposes***(Sharma etal. 2010;Singh etal 2013).***

2. Most of the literature is limited towards case reports, providing no evidence of predictability and few demonstrate long term stability. No controlled clinical trial has addressed the issue of restoring deficient interproximal spaces ***(Carnio 2004).***

3. Nonsurgical techniques are currently considered superior to conventional surgeries regarding decreasing the surgical trauma, improving the clot stability, reducing patient discomfort postoperatively as well as minimizing surgical chair time ***(Cortellini 2001)*** Based on theses principles, nonsurgical injection of hyaluronic acid seemed to overcome the major limiting factor in most surgical techniques regarding the limited blood supply and the small working space.

4. Supported by a recent study conducted by ***Georgieva etal. 2017***, who reported an increase in the height of the gingival papilla and reduction of the area of the black triangle that were significant between baseline and 3 or 6 months in a group with thick gingival biotype after injection with hyaluronic acid, Yet maximum papillary full gain effect is limited to other factors.

5. Least traumatic surgical attempts were presented aiming at full papilla reconstruction and are based on Sub-epithelial connective tissue graft for the beneficial genetically determinant factors it possess guiding gingival tissue for regeneration yet was lacking flap design precision to provide wound stability to fulfill full gain benefit ***(Carranza & Zogbi 2011).***

6. Based on principles needed to preserve connective tissue graft, a case report has tested a novel technique {Tube} for treating interproximal recession and reconstructing the interproximal papilla, resulted in uneventful healing at both the donor and recipient sites in

both cases. With a net gain of 5 mm measured one year post-surgically ***(Hooshang etal 2020).***

Review of literature:

The interdental papilla is the gingival portion that fills the proximal area under the contact between two adjacent teeth, where it is defined as gingival tissues extending from incisal tip of the papilla to a line tangential to the adjacent margins of the adjacent teeth. Anatomically, it is small and delicate with a diminutive blood supply, it is almost the most important esthetic part during smiling. ***(Csiszar etal.2007).***

Nordland & Tarnow 1998 classified papillary loss according to system which utilizes 3 identifiable anatomical landmarks: the interdental contact point, the facial apical extent of the cemento-enamel junction (CEJ) and the interproximal coronal extent of the CEJ. Normal Interdental papilla fills embrasure space to the apical extent of the interdental contact point/area. Class I which the tip of the interdental papilla lies between the interdental contact point and the most coronal extent of the interproximal CEJ (space present but interproximal CEJ is not visible). Class II which the tip of the interdental papilla lies at or apical to the interproximal CEJ but coronal to the apical extent of the facial CEJ (interproximal CEJ visible). Class III which the tip of the interdental papilla lies level with or apical to the facial CEJ.

The absence of interdental papilla contributes to chronic retention of food debris leading to subsequent affection of periodontal health and esthetically unaccepted black triangle***(Lee etal.2016).***The etiological pyramid of the black space has been proposed by ***Sharma and Park in 2010***. To be influenced by the initial position of the teeth (diastema), the length of gingival niche area, triangular shaped crown, divergent roots and periodontal bone loss. Also tooth brush trauma and decreased keratinization due to ageing has also been implicated as a causative factor of interdental papilla loss.

Reconstruction of papillary insufficiency is one of the most difficult and challenging periodontal treatments. This is because the interdental papilla is a small, fragile area with minor

blood supply which seems to be the major limiting factor in all surgical and augmentation techniques ***(Singh etal. 2013;Al-Zarea etal. 2015).***

The injection of biocompatible hyaluronic acid gel (HA) have been considered conventional non-invasive techniques for treatment of deficient papilla. Hyaluronic acid (0.1) mm It is an essential glycosaminoglycan of the extracellular matrix of the periodontal tissues, and the majority of cells can produce it during several phases of their cell cycle. It is involved in tissue repair and wound healing by stimulating cell proliferation, migration and interaction with several growth factors. Furthermore, HA has a crucial role in space-filling owing to its hygroscopic nature. Moreover, it regulates osmotic pressure and enhances tissue lubrication and resiliency, which helps in maintaining the structural and homeostatic integrity of tissues ***(Ascher etal. 2011).***

However, hyaluronic acid gel injection still entails unpredicted complete gingival papilla improvement owing to the relative compact nature of the gingiva and influence of the gingival biotype that could hinder the positive outcomes on favor of thick biotype that could help to enrich the effect of hyaluronic acid through accelerating the thick connective tissue related abundance of gingival fibroblast proliferation, promoting the generation of collagen fibers. Limited studies have utilized HA gels for papillary reconstruction but still lacking a high degree of evidence and predictability ***(Becker etal. 2010; Mansouri etal. 2013)***

Several conventional surgical approaches using traditional periodontal plastic and augmentation procedures aiming for complete papilla fill to overcome this problem. However, flap design were found to be invasive with increased patient morbidity, limited success, long-term stability and unpredictability due to small working spaces and limited blood supply. ***(Georgieva, etal. 2017;Alahmari 2018).***

Most of the surgical approaches targeting papilla reconstruction were based on Sub-epithelial connective tissue graft **(*Beagles etal 1996). Han & Takei 1996*** used semilunar coronally repositioned papilla based on the use of free connective tissue graft. He concluded that subsequent second- and possibly third-stage surgery should reconstruct more of the papilla which is considered one of the disadvantages of this flap design. So ***Pellegrine 1996*** modified this technique by folding the CTG to provide adequate thickness of the attached gingiva.

Modified Beagle's technique and Beagle's surgical technique were unpredictable due to

small working spaces and limited blood supply to the area in addition to the vertical releasing incisions that could further jeopardize vascular supply and leave unpleasant scarring after healing ***(Beagles 1996;Chaulkar 2020).***

Han and Tackei technique made a trial to modify the flap design by semilunar coronally repositioned papilla that enabled to preserve the interdental papilla blood supply and improving the gingival biotype from 0.75 mm to 2 mm, One year post-operative view showing 80% papillary fill ***(Jangid etal.2016***).

These subepithelial conventional techniques were unpredictable due to small working spaces and limited blood supply to the area. Disadvantages of these techniques were mostly due to the number and location of incisions, which could have disrupted the blood supply needed for complete healing***(Azzi etal. 2001).***

Microsurgical techniques had been introduced and enhance the visual accuracy by means of illumination and magnification. Literature on periodontal microsurgery showed improved surgical outcome when used for root coverage procedure and regenerative therapy. ***(Wachtel etal. 2003; Francetti. etal.2005).*** By the aid of subepithelial connective tissue, the microsurgical technique found to maintain the reconstructed papilla remained stable and without any clinical signs of inflammation for 4 years after surgical procedure, but the long-term survivability and the technique sensitivity involved in the surgery are to be considered ***(Nordland 2008).***

Recent clinical study assessed the efficacy of microsurgical reconstruction of lost interdental papillam and was based on single surgical intervention, with the aid of connective tissue graft which is proved useful in Correction of papillary loss, but the Maximum gain with single procedure was found to be limited. Other limitations of the study were being due to smaller sample size and short period of follow up and were recorded for long term clinical and histological follow up studies are necessary before its predictability ***(Singh etal. 2018).***

Tube technique is recently introduced by ***Hooshang etal. 2020*** on the track of surgical techniques to overcome disadvantages of the other techniques. The innovated flap design of tube technique does not disturb the blood supply of the graft by its use of apical incisions and containment of the graft within a rich vascular enclosed bed, thereby offering reliable and

satisfactory results. Integrity of the soft tissue is key to the successful outcome of this technique. The tube grafting technique requires technical precision. It is sensitive to any surgical trauma and tension, especially when the recipient site tissue is delicate.

Thus, the present randomized clinical trial is to compare the clinical effect of surgical Tube technique versus Hyaluronic Acid Gel injection in Reconstruction of the interproximal papilla in patients with class II papillary defect.

Comparator: [Standard of care] Hyaluronic Acid injection

It is a minimally invasive non-surgical injection of hyaluronic acid that observed to overcome the major limiting factor in most surgical techniques regarding the limited blood supply and the small working space. Supported by a recent study conducted by ***Jinng etal. 2019***, who reported significant increase in the height of the gingival papilla and reduction of the area of the black triangle between baseline and 3 or 6 months in a group with thick gingival biotype after injection with hyaluronic acid, that was adding possible maximum effect with minimal postoperative hazards.

II.B. Objectives of the study:

7. Aim of the study

The aim of this randomized clinical trial is to compare the clinical effect of surgical Tube technique versus Hyaluronic Acid gel injection in reconstruction of the interproximal papilla in patients with class II papillary defect.

Picots:

P: Population: patients with class II papillary defect.

I: Intervention: Tube technique hosting sub-epithelial connective tissue graft.

C: Control: Hyaluronic acid injection.

Primary outcome: interdental papilla loss (Decrease of black triangle) (mm2)

Secondary outcome: assessment of gain in papillary height , radiographic distance between (CP-BC), assessment of CAL. PD reduction, amount of gingival recession.

T: Time frame: 6 months.

S: Study design: Randomized clinical trial.

Hypothesis:

Null hypothesis: There is no difference in the gain in papillary height comparing hyaluronic acid injection versus Tube technique in patients with class II papillary defect.

8.Trial design

The study is designed as a superiority, 2 arm parallel groups, randomized clinical trial with allocation ratio 1:1.

III. Methods

A) Participants, interventions and outcomes

9. Study settings:

This study will be carried out on patients enrolled from the outpatient and postgraduate clinics of Oral Medicine and Periodontology department, Faculty of Dentistry, Cairo University.

10. Eligibility criteria:

Inclusion Criteria:

- Highly motivated patients with papillary deficiency types II, according to ***Nordland and Tarnow 1998*** classification will be selected, having at least one deficient papilla in the anterior region.
- Distance between the contact point and inter-proximal bone crest (CP-BC) of ≤ 7 mm and probing depth of ≤ 4 mm at the deficient papillary site ***(Abdelraouf etal. 2019)***.
- Patients with full mouth plaque index (PI) and gingival index (GI) scores should be between 0-1.
- Healthy patients free of any medical condition.

Exclusion criteria:

- Patients with medical conditions that may affect periodontal healing or regeneration.
- Patients with a history of allergic reactions, pregnant or breastfeeding females, smokers and alcoholics.
- Patients with current or previous drugs intake that may predispose to gingival enlargement.
- Patients under orthodontic treatment or had orthodontic treatment in the past six months.
- Carious teeth, proximal restorations or fixed prosthesis.
- Patients with a history of traumatic oral hygiene measures or periodontal surgeries over the last six months at the area of interest or having any scar tissue.
- Poor oral hygiene patients.

11. Interventions

11a-General operative procedure

- The patients who fulfil the inclusion criteria will be enrolled.
- The nature of the study will be explained to each patient as well as the importance of compliance with pre- and post-operative instructions and follow-up visits.
- Each patient will be asked to sign an informed consent (appendix 1)
- After enrolment, periodontal examination and full periodontal charts will be recorded for each patient including Papilla Class I1, CP-BC plaque Index ***(Leary et al., 1967)***, gingival bleeding Index ***(Ainamo & Bay, 1975)***, probing depth (PD), clinical attachment level (CAL) and gingival recession.

Radiographic Evaluation

Standardized digital periapical radiographs will be obtained preoperatively and at follow-up using the long cone paralleling technique. All radiographs will be obtained using the same equipment, film exposure and development conditions.

Pre-surgical therapy

- All patient will receive strict oral hygiene instructions full mouth supra and sub-gingival debridement using hand instruments and ultrasonic scalers under local anesthesia.
- Four to six weeks following pre-surgical therapy, a periodontal re-evaluation will be performed to confirm the suitability of the chosen sites for this periodontal surgical study.
- The clinical and radiographic parameters will be recorded at baseline

The patients will be randomly assigned into two groups: **A total of 36 sites (18 site per group)**

Group A (Intervention): Patients will receive Tube technique.

Group B (Control): Patients will receive Hyaluronic acid.

Surgical Procedures

Group A (Intervention): Patients will receive Tube technique ***(Hooshang etal. 2020).***

1) Using a #15 blade, a semilunar incision is placed on the buccal aspect at the level of the mucogingival junction and a full-thickness flap was elevated. The flap is extended mesio-distally by an additional 4 to 5 mm than needed for the graft. This extension allowed the buccal flap to be freely moved into the recession area.

2) Another semilunar incision will be placed on the palatal aspect, such that the zenith of the

incision is located proximal to the alveolar crest.

3) An excavator (smaller than the width of the defect; evaluated pre-surgically on a study model) is used to carefully reflect the papilla away from the alveolar bone and root surfaces on the buccal aspect to access the interproximal region to prepare the bed for the graft. The excavator is used to do the same on the palatal aspect. The amount of reflection should yield a very relaxed papilla flap that permits placement of the papilla at the new coronal position without tension.

4) The recipient site at this time should resemble a "tube" with two openings; one on the buccal aspect at the mucogingival junction and one on the palatal aspect.

5) To harvest the graft, the incision is made parallel to and 2 mm away from the gingival margin. A thin flap containing the keratinized tissue is separated, then the second incision is made parallel to the gingival margin, but perpendicular to the alveolar bone. With a sharp periosteal elevator, the graft is raised with the periosteum and released at its anterior and posterior ends, and then from its base .The harvested graft should be larger than the side of interproximal defect in both cases: each graft will be 12 to 15 mm long, 5 mm wide, and 2 mm thick. A 4.0 chromic gut suture is used to pass the eye (swage) of the needle into the tube from the palate toward the buccal. The suture needle then engaged one end of the graft. An additional separate second suture is placed on the other end of the CT graft. One suture end (eye first) is passed through the tube toward the palatal side

6) The connective tissue graft is carefully slipped through the buccal aspect of the tube into the prepared interproximal bed towards the palate by means of the suture with the assistance of a periosteal elevator as needed. The thickest part of the graft is placed in the interproximal area.

7) The connective graft is sutured under the flaps onto the buccal and palatal tissues and the buccal and palatal flaps are sutured over the top of the graft.

8) The size of the needle does not matter for this procedure; we use 4-0 silk with an FS2 cutting needle and a 4-0 chromic gut suture with a C-6 (FS-2) cutting needle.

9) Using the index finger and thumb the graft will be squeezed from both ends inward for at least 4 to 5 minutes to further stabilize the graft into position. Both patients are instructed to avoid

brushing or flossing the recipient as well as donor site. They are also asked to avoid hot, chewy, sticky foods. They were advised to ice the wound to reduce post-surgical swelling.

Group B (Control): Patients will receive Hyaluronic acid (HA) (0.1) mm ***(Abdelraouf etal. 2019)*** The product used in this trial is Restylane Lidocaine (Restylane-Lidocaine cross-linked Hyaluronic Acid Filler, Galderma S.A, Sweden). Restylane was the first FDA-approved HA filler in 2003. Restylane is a non-animal stabilised cross-linked HA filler with an HA concentration of 20 mg/ml. The longevity of Restylane filler in tissues is approximately 6 months. Restylane-Lidocaine (FDA approved in 2012) is a newer product of Restylane with 0.3% lidocaine incorporated into the syringe itself.

-Stent will be performed and re evaluation

-After 4 weeks, re-evaluation is performed, and the degree of papillary deficiency according to ***Nordland and Tarnow*** **1998** classification as well as plaque and gingival indices will be assessed for eligibility. Alginate impression is taken for the involved arch/arches for the construction of study casts and fabrication of customized stents. Only patients with deficient papilla sites fulfilling the inclusion criteria are recalled, scheduled for the 1st injection after 1 week and will sign the informed consent.

After 1 week 3 injection will be given at each papilla site

-In the injection phase, 3 injections are given at each papilla site: at baseline, 3- and 6-weeks intervals. At the first injection visit, before attempting to inject, clinical measurement of the height of the black triangle is done by measuring the distance between the deficient papilla tip and contact area (PT-CP distance) to the nearest 0.5 mm (baseline). This will be done using a graduated periodontal probe and the fabricated customized stent for proper and standardized positioning of the probe at each measurement interval

- Hyaluronic acid and saline are pre-loaded in insulin plastic syringes before injection for patient blinding. The needle is inserted 2-3 mm apical to the tip of the interdental papilla and directed coronally with an angulation of 45° to the long axis of the tooth, and the bevel directed apically. Then, the papilla is lightly moulded in an incisal direction for 1 minute using gauze.

-In the follow-up phase, patients are recalled after 3,6months the first injection where clinical re-measurement of the black triangles and standardized digital clinical photographs will be retaken.

Postoperative care:

-Patient self-care instructions (Heitz et al. 2004):

- Avoid any hard brushing and trauma to the surgical site for two weeks.
- Maintain a soft diet to avoid trauma to the surgical site.
- After 24 hours patients can use warm saline rinses daily
- Sutures will be removed fourteen days after the surgery.
- Plaque control will be maintained by chlorohexidine mouth rinse after the surgery.

12. Outcomes:

Outcomes	Outcome name	Measuring device	Measuring units
1ry outcome	interdental papilla loss (Decrease of black triangle area) (mm2)	Photoshop CS5.	mm
2nd outcomes	Gain in papillary height Change in the distance (CP-BC) CAL Pocket depth (PD) Gingival recession (GR) Gingival bleeding Plaque index Post-surgical patient Satisfaction and post-injection	UNC Periodontal Probe Digital periapical radiograph (linear measurement) UNC Periodontal Probe UNC Periodontal probe UNC Periodontal probe UNC Periodontal probe UNC Periodontal probe Post-surgical patient satisfaction questionnaire (PSPSQ) (Kiyak, Hohl, West, & McNeill, 1984)	mm mm mm mm mm Gingival bleeding score Plaque Index score (1-7) point scale

-Digital clinical photographs: (SABT) mm ***(Abdelraouf etal. 2019)***

Clinical photographs will be obtained with the same digital camera (Nikon D5100 DSLR) mounted on a ring flashlight, using the same lens (Sigma Macro 105mm F2.8 EX) and the same focal length. The photographs are taken under the same lightning conditions and camera settings. The patients are sitting in an upright position, looking straight ahead. The Frankfort plane of the patient as well as the camera lens is positioned parallel to the ground. The photographs will be captured perpendicular to the teeth adjacent to the deficient papilla. Strict care is taken to ensure that the same up-down and right-left shooting positions are reproduced at different time intervals.

-The surface area of the black triangle (SABT) is assessed using standardized digital clinical photographs analyzed by an image analysis program (Photoshop Cs 5, Adobe Systems, San Jose, CA, USA). The surface area of the black triangle was assessed from the photographs taken at baseline (before injection), 3 and 6 months from the first injection.

- 10 mm William's graduated periodontal probe was used as a scale for calibration.

Papilla Presence Index (PPI): ***(Cardaropoli etal. 2004)***

-PPI (1): is reported when papilla is completely present and coronally extends to the contact point to completely fill the inter-proximal embrasure which this papilla is at the same level as the adjacent papillae.

-PPI (2): describes a papilla that is no longer completely present and lies apical to the contact point which this papilla is not at the same level as the adjacent papillae and the embrasure is no longer completely filled but the interproximal cemento-enamel junction is still not visible.

-PPI (3): refers to the situation in which the papilla is moved more apical and the cemento-enamel junction becomes visible.

-PPI (4): describes when the papilla lies apical to both the incisal cemento-enamel junction and buccal cemento-enamel junction which interproximal soft tissue recession is present together with buccal gingival recession and patients esthetics is dramatically compromised.

Clinical attachment level (CAL):

CAL will be determined by measuring the distance from the cemento-enamel junction to the base of the pocket using a UNC 15 periodontal probe.

Probing depth (PD):

Probing depth will be measured from the gingival margin to the base of the periodontal pocket using a UNC 15 periodontal probe.

Gingival recession (GR):

Gingival recession will be measured using UNC periodontal probe from the cemento-enamel junction till the gingival margin ***(Miller, 1985).***

Gingival Bleeding score:

- Gingival bleeding score will be performed through gentle probing of the orifice of the gingival crevice from all four surfaces of all teeth using UNC Periodontal probe.
- Recorded as (+) if bleeding is present or (-) if bleeding is absent.
- If bleeding occurs within 10 seconds a positive finding is recorded and the number of positive sites is recorded and then expressed as a percentage of the number of sites examined (***Ainamo & Bay, 1975***).

Patient Satisfaction:

- Three questionnaires will be asked to determine patients' satisfaction with the outcome of surgery
  1. Considering this was an elective operation, how likely would you be to recommend it to others?
  2. If you had to make the decision again, how likely would you be to undergo this surgery'?
  3. Considering everything, how satisfied are you with the outcome of your surgery
- These questions could all be answered by the patient using a 7-point response scale where: Very Satisfied = 7; Not At All Satisfied = 1. ***(Kiyak et al., 1984).***

13. Participant timeline

13. Participant timeline Time point	T0	T1	T2 (Surgical phase- 8 weeks)	T3 Starting from T2 till 7th day	T4 3-months post-surgical	T5 6-months post-surgical	1-
Enrollment	X						
Eligibility screen	X						
Informed consent	X						
Initial phase (Oral hygiene measures) standardized periapical radiograph study casts	X	X				X	
Allocation			X				
Intervention Injection Surgical tube technique			X				
Post-operative Care				X			
Papillary height , CAL, PD, GR measurements		X	X		X	X	

SABT							
Patient Satisfaction			X			X	

14. **Sample size:**

This power analysis used interdental papilla loss after six months as the primary outcome. Based upon the results of Awartani FA and Tatakis DN (2016), the mean and standard deviation (SD) for control group were 0.71 (0.74) mm2. The minimal clinically significant difference was 1 mm2according to expert opinion. Using alpha (α) level of (5%), β level of 0.8 (Power = 80%); the effect size for independent samples t-test (d) was 1.32 and the minimum estimated sample size was 10 sites per group. Sample size was increased to 12 sites per group to compensate for a drop-out rate of 20% after six months. Sample size calculation was performed using PS Power and Sample Size Calculations Version 3.

15. Recruitment strategy

- The main investigator will be responsible for recruitment of patients.
- Patients will be selected from the outpatient clinic of the Oral Medicine and Periodontology Department, Faculty of Dentistry - Cairo University.
- Screening of patients will continue until the target sample is achieved.
- Identifying and recruiting potential subjects will be achieved through patient database.

1. One year.
2. Candidate self-funding

B) Assignment of interventions

16. Allocation

16a. Randomization

The patients will be randomly allocated to either the intervention group or the control group using computer generated random numbers that will be performed by the supervisor. The patients will be allocated in a ratio of 1:1.

16b. Allocation concealment mechanism

The two groups will be equally prepared for both surgical procedures. Then the decision of which group will receive Hyaluronic acid injection and which will Tube Technique will be taken according to the randomized numbers placed in opaque sealed envelopes. The number will be picked by the supervisor.

16c. Implementation

The supervisor will be responsible for generation of allocation sequence as will as assign the patients to the 2 intervention groups. The main investigator will be responsible for patient enrollment.

17. Masking/blinding:

Due to the type of intervention only the outcome assessor and the statistician will be blinded.

Single blinded:

- Blinding of the participants is not applicable.
- Blinding of the operator is not applicable.
- Outcome assessor (primary and secondary outcomes) and biostatistician will be blinded.

Key elements of Random Sequence Generation in protocol are:

1-Method of random sequence generation (computerized random number generator).

2- Allocation ratio (1:1, 2:1).

3- Type of randomization: simple, blocked, stratified.

c) Data collection, management, and analysis:

18. Data collection methods

- All the data of the patients included in the study will be recorded from them or extracted from the patient's periodontal chart.

- Telephone numbers of all patients included in the study will be recorded as a part of the written consent.
- All patients will receive a phone call at the time of the pre-determined follow up dates.

19. Data management:

Excel sheet program will be used though a computer system to manage the data with a secured password.

20. Statistical methods:

- The outcome used (State 1ry or 2ry): interdental papilla loss area (mm2)
- Values used for outcome (e.g. mean difference, percentage, proportion..... etc): mean and standard deviation
- Entry 1: (mean difference between 2 groups, mean and standard deviation of group 1, proportion 1 or estimated prevalence):

Minimal clinically significant difference = 1 mm2 according to expert opinion

- Entry 2: (standard deviation of control group, mean and standard deviation of group 2, proportion 2 or clinically important difference):

Mean of the control group = 0.71

SD of control group = 0.74

- Alpha level of significance: 0.05
- Effect size used in calculation: d = 1.32
- Power of the study: 0.8
- Statistical test used: Independent samples t-test
- The calculated sample size: 10 sites per group.
- Increased number for anticipated missing data: Sample size will be increased to 12 sites per group to compensate for a drop-out rate of 20% after six months.

D) Data monitoring:

21. Monitoring

No formal data monitoring committee will be needed since it is a study with known minimal risks.

22. Harms

Postoperative pain and/or swelling will be managed through analgesics.

23. Audit

Auditing of the study design will be done by the evidence-based committee – Faculty

Dentistry – Cairo University.

IV. Ethics and dissemination

24. Research ethics approval

This protocol and the template informed consent form will be reviewed by the Ethics Committee of Scientific Research -Faculty of Dentistry- Cairo University.

25. Protocol amendments

Any modifications to the protocol which may have an impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendment will be agreed upon by the Council of Oral Medicine and Periodontology Department and the Faculty of Dentistry, Cairo University.

26. Informed consent

Researcher will introduce the trial to patients and provide full explanation of its aim and benefits in layman language. Patients will then be able to have an informed discussion with the researcher. Researcher will obtain written consent from patients willing to participate in the trial. All consent forms will be translated into Arabic.

27. Confidentiality

All study-related information will be stored securely. All participant information will be stored in locked file cabinets in areas with limited access. All reports, data collection, process, and administrative forms will be identified by a coded ID [identification] number only to maintain participant confidentiality. All records that contain names or other personal identifiers will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems.

28. Declaration of interest

If there will be any conflict of interest it will be declared.

29. Access to data

The principal investigator and the supervisors will have access to the data sets. All data sets will be password protected. To ensure confidentiality, data dispersed to project team members will be blinded of any identifying participant information.

30. Post-trial care

All patients will be followed up for six months after the clinical trial is over.

31. Dissemination policy

- Study results will be published as partial fulfillment of the requirements for Doctorate's degree in Periodontology. - Topics suggested for presentation or publication will be circulated by the authors.

32. Informed consent

VI. Statement of originality

To the best of our knowledge, no randomized clinical trial has been published that involve comparing the clinical effect of surgical tube technique to hyaluronic acid gel injection in reconstruction of the tnterproximal papilla in patients with class II papillary defect.

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