Soft tissue reconstructive techniques at implant sites: a clinical, volumetric and ultrasonographic analysis

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PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

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LIST OF ABBREVIATION

AMW: attached mucosa width
BBD: buccal bone dehiscence
BBT: buccal bone thickness
CAF: coronally advanced flap
CAL: clinical attachment level
CBCT: cone beam computed tomography
CM-CB: Distance between the crown margin and the crestal bone
CM-STM: Distance between the crown margin and the soft tissue margin
CP: ultrasonographic color power
CV: ultrasonographic color velocity
CTG: connective tissue graft
eCAF: envelope coronally advanced flap
CAF: coronally advanced flap
FGG: free gingival graft
GPF: greater palatine foramen
IDES: Implant soft tissue Dehiscence coverage Esthetic Score
KMW: keratinized mucosa width
MGJ: mucogingival junction
MT: mucosa thickness
PD: probing depth
PSP: peri-implant soft tissue phenotype
PSTD: peri-implant soft tissue dehiscence
STH: supracrestal tissue height
TUN: tunnel technique
UMT: mucosal thickness measured with ultrasound
US: ultrasound

1. INTRODUCTION

1.1 The peri-implant phenotype

Dental implants have shown to be a reliable tool for single, multiple and full-arch rehabilitations [1]. Dental implants have a very high success rate in terms of osseointegration, however biological, prosthetic and esthetic complications are not rare . While the significance of peri-implant bone volume and the necessity of performing bone augmentation if deficient, has been extensively discussed [2, 3], the critical role of peri-implant soft tissue on implant esthetics and health has also been the topic of significant discussion in the last decade [4].

The peri-implant phenotype has been defined recently by Avila-Ortiz et al. as the morphologic and dimensional features characterizing the clinical presentation of the tissues that surround and support osseointegrated implants [5]. The peri-implant phenotype encompasses a soft tissue component, which includes the peri-implant keratinized mucosa width (KMW), the mucosal thickness (MT) and the supracrestal tissue height (STH), and an osseous component, characterized by the peri-implant bone thickness (BBT). This definition does not only apply to buccal and facial sites, but also to lingual and palatal peri-implant locations. Like the periodontal phenotype [6], the peri-implant phenotype is site-specific and may change over time in response to environmental factors [5].

Peri-implant keratinized mucosa width is the height of keratinized tissue in an apico-coronal direction between the soft tissue margin and the mucogingival junction (MGJ). KMW may be completely absent in certain cases in which there is only alveolar mucosa surrounding the implant(s) [5]. While several investigators have shown that an insufficient KMW around dental implants is associated with more plaque accumulation, tissue inflammation, mucosal recession and attachment loss [7-11], others have failed to reach such conclusions [12-14]. However, recent evidence seems to suggest that KMW plays a protective effect on peri-implant tissues. In a 10-year prospective study, Roccuzzo et al. observed significantly greater plaque accumulation and deeper mucosal recession (peri-implant soft tissue dehiscence [PSTD]) for implants without KMW. In addition, more sites from the group of implants without KMW required additional treatment, including surgeries with free gingival graft (FGG) or antibiotics. Patients with implants without KMW that received FGG reported reduced discomfort and showed better plaque control [15]. Souza et al. confirmed that KMW plays a role on patient brushing comfort [16]. More recently, a 4-year prospective study by Perussolo and coworkers showed that implant with narrow KMW width (< 2 mm) had higher level of brushing discomfort, plaque index and bleeding on probing than implants with wide KMW (≥ 2 mm). In addition, implants with narrow KMW were found to have higher marginal bone loss, leading the authors to conclude that KMW width ≥ 2 mm may have a protective effect on peri-implant tissues [9]. In a cross-sectional study, it was found that reduced KMW width is a risk indicator for the severity of peri-implant mucositis, [7] and in line with this finding, Schwarz et al. concluded that KMW plays a role on the prevention and resolution of peri-implant mucositis [17]. Furthermore, the absence of peri-implant KMW has also been related to lower patient esthetic satisfaction [18], verifying the importance of the soft tissue component on implant esthetics [19, 20].

Oh and coworkers showed that FGG is a predictable option for increasing KMW around dental implants, with benefits in terms of lower inflammatory index and better marginal bone

level stability compared to implants that did not receive FGG for up to 48 months [21, 22]. A recent systematic review and network meta-analysis from our group further confirmed that APF + autogenous free gingival graft (FGG) is the gold standard technique for increasing KMW at implant sites [23]. Nevertheless, patient morbidity, the need for a second surgical site, limited availability and increased surgical time are the main disadvantages of FGG, that led clinicians to explore alternatives approaches and graft materials, including xenogeneic collagen matrices and acellular dermal matrices [24-27]. Although these graft substitutes showed promising results when utilized with bilaminar techniques [26-28], they demonstrated to have limited efficacy when combined with APF for increasing/regenerating KMW [23, 29, 30].

It has been suggested to use xenogeneic collagen matrix in combination with a small autogenous graft (strip gingival graft), which is suture apically to the graft substitute, providing a source of autogenous cells that can migrate into the collagen scaffold to regenerate the lost KMW [31-33].

Peri-implant mucosal thickness is the horizontal dimension of the peri-implant soft tissue, which may or may not be keratinized. MT may vary at different location and apico-coronal heights respective to the mucosal margin around a given implant [5]. Soft tissue augmentation for increasing MT is mostly intended to improve esthetic outcomes and to compensate for volume deficiencies [20, 34-36]. Nevertheless, there is no consensus regarding the required amount of MT [37]. Jung and coworkers showed that when MT is < 2 mm, the choice of abutment material strongly influences esthetic outcomes [38]. Similarly, several studies have found a correlation between MT exceeding 2 mm and higher esthetic outcomes [39, 40]. Therefore, it's not surprising that one of the most common indications for MT augmentation is the attempt to attenuate or eliminate the effect of the shade of the abutment on the buccal aspect of the mucosa and/or to compensate for possible underlying bone deficiencies prior or after functional loading [5]. A thicker peri-implant soft tissue can also provide greater marginal stability than thin MT [41-43], which is considered to be one the main factors associated with mucosal recession [43-45].

Whether MT plays a role on peri-implant health has been controversial over the years. According to Thoma et al., soft tissue augmentation with autogenous grafts may result in significantly less MBL. Similar results were found by Puzio and coworkers, demonstrating that PSP modification with bilaminar techniques (either connective tissue graft [CTG] or xenogeneic collagen matrix) is recommended when MT is < 2.88 mm. On the other hand, a recent 5-year prospective study comparing implants with or without CTG found a better subjective evaluation of mucosa color in the grafted sites, without observing any differences in the marginal bone levels. Results from our recent meta-analysis demonstrated that peri-implant soft tissue phenotype modification with CTG or xenogeneic collagen matrix showed beneficial effects on marginal bone level stability [23].

The peri-implant supracrestal tissue height is the vertical dimension of the soft tissue that surrounds a dental implant from the mucosal margin to the crestal bone [5]. Different from KMW width and MT, STH can be assessed circumferentially around an implant, including proximal sites. In a corono-apical direction, the peri-implant STH encompasses the sulcular epithelium, the junctional epithelium, and the supracrestal connective tissue, which is typically

not attached to the abutment surface [5]. In a series of studies from Linkevicius et al., it was demonstrated that a thin peri-implant mucosa, as measured from the bone crest in an apico-coronal direction, also referred to as the supracrestal tissue height is associated with higher marginal bone loss than a thick tissue phenotype, and that augmenting STH with a soft tissue graft was effective in minimizing peri-implant bone loss [46, 47]. A systematic review and meta-analysis from Suarez-Lopez del Amo concluded that implants placed with an initially thicker MT have less radiographic marginal bone loss in the short term [48]. Similarly, a more recent article corroborated that the association between thin STH and higher marginal bone loss, especially for crestally-positioned implants [49].

Peri-implant bone thickness is the horizontal dimension of osseous tissue that supports an osseointegrated implant. BBT may vary at different apico-coronal heights respective to the bone crest around a given implant or even be completely absent in sites exhibiting peri-implant bone defects (e.g., fenestrations or dehiscences). According to Thoma et al., vertical bone defect, such as dehiscence, resolution seems to be more important than the horizontal bone thickness at the level of the implant shoulder [50]. Based on a large prospective study, Spray and colleagues observed that implants having at least 1.8 mm 0.5 mm apical to the crest at the time of implant placement had a lower rate of vertical bone loss [2].

1.2 Soft tissue deformities at implant sites

Dental implants can be characterized by several soft tissue deformities, including lack of KMW, attached mucosa, and/or inadequate MT. Other deformities include the level of the soft tissue margin, strictly related to implant esthetic complications. A discrepancy in the level of the peri-implant soft tissue compared to the level of the gingival margin of the contralateral adjacent tooth has been defined peri-implant soft tissue dehiscence (PSTD)[43, 44].

1.2.1 Definition of peri-implant soft tissue dehiscences

The success of implant therapy should not be solely dependent upon its long-term survival, but also on its functional, esthetic, hard and soft tissues stability, as well as patient reported outcomes [51]. Indeed, over the years patients' esthetic demands have increased such that even a minimal apical shift of the gingival margin revealing the greyish color of the implant may be considered unacceptable, especially in the esthetic region [44, 52]. An apical shift of the peri-implant facial soft tissue margin has been defined with many terms throughout the literature, including mid-facial recession, mucosal recession or dehiscence, soft-tissue dehiscence/deficiency or a soft-tissue defect [44]. As these complications can manifest either as mucosal recessions (apical shifting of the peri-implant mucosal margin with respect to the homologous natural tooth with or without exposure of the metallic part of the implant), or only a greyish hue noticeable through the mucosa, the term PSTD may be the most appropriate for their description [43, 44].

1.2.2 Novel Classification of Peri-implant soft tissue dehiscences/deficiencies at single implant site in the esthetic zone

Zucchelli et al. has recently published a novel classification system describing PSTDs at single implant site in the esthetic zone [43]. This classification focuses on healthy dental implants,

characterized by an esthetic complication and not affected by peri-implant diseases. In agreement with the 2017 World Workshop [53], diagnosis of peri-implantitis made - in the absent of baseline radiographs – based on signs of inflammation on gentle probing, probing depth of 6 mm or more and bone levels ≥ 3 mm apical of the most coronal portion of the intra-osseous part of the implant. In the presence of previous examination data, a peri-implant disease is defined based on presence of bleeding on probing, increasing probing depth compared to previous examinations and the presence of radiographic bone loss beyond crestal bone level changes resulting from initial remodeling [53]. For implants diagnosed with peri-implant mucositis or peri-implantitis, these conditions must be addressed prior to applying this novel classification of PSTDs, which also aims at providing guidelines and recommendations for treatment.

The new classification of PSTD involved the identification of classes and subclasses. The PSTD class is related to the apico-coronal position of the soft tissue margin and the bucco-palatal position of the implant, while the subclass reflects the height of the interproximal soft tissue/peri-implant papillae (Figure 1).

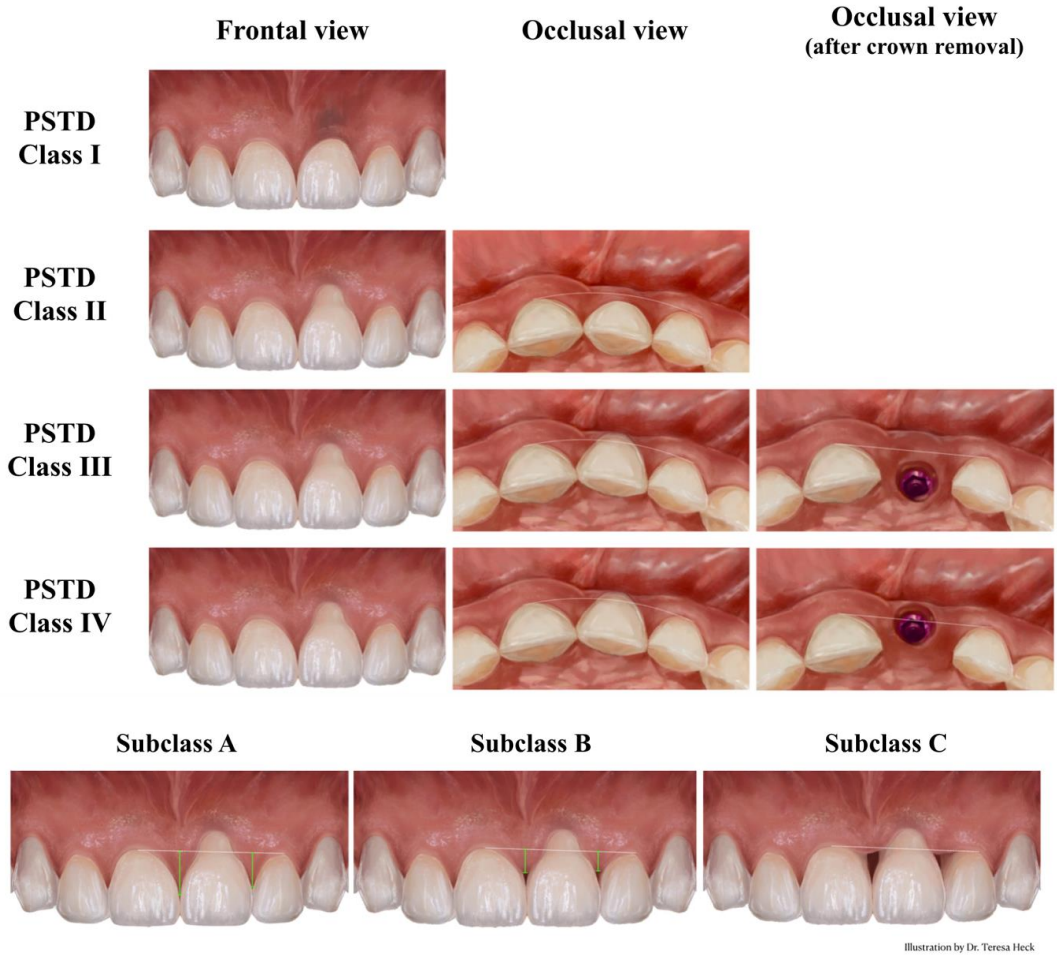
- **Class I**. The soft tissue margin is located in an esthetically correct position (at the same level of the ideal position of the gingival margin of the homologous natural tooth), and the color of the abutment/implant is visible only through the mucosa and/or there is a lack of keratinized tissue/soft tissue thickness
- **Class II**. The soft tissue margin is located more apical to the ideal position of the gingival margin of the homologous natural tooth and the implant-supported crown profile is located inside (more palatal) the imaginary curve line that connects the profile of the adjacent teeth at the level of the soft tissue margin.
- **Class III and IV**. The soft tissue margin is located more apical to the ideal position of the gingival margin of the homologous natural tooth and the implant-supported crown profile is located outside (more facial to) the imaginary curve line that connects the profile of the adjacent teeth at the level of the soft tissue margin. In these classes it is mandatory to remove the implant-supported crown. When the head of the implant is inside (more palatal or at the level of) the straight imaginary line that connects the profile of the adjacent teeth at the level of the gingival margin, the PSTD is defined as Class III, while when the implant head is outside (more facial) this imaginary line, this is referred to as Class IV.

Each of the classes (except for Class I where subgroup c is not clinically detectable) can be further sub-divided into the following subcategories in relation to the papilla dimension:

- **Subclass a**: when the tip of both papillae is ≥ 3 mm coronal to the ideal position of soft tissue margin of the implant-supported crown
- **Subclass b**: when the tip of at least one papilla is at a distance < 3 mm coronal to the ideal position of the soft tissue margin of the implant supported crown
- **Subclass c**: when the height of at least one papilla is at the same level or more apical of the ideal position of the soft tissue margin of the implant-supported crown

This classification has shown to be reliable and reproducible also dental practitioners with different skill levels and expertise [54].

Figure 1. Illustration of the 4 classes of peri-implant soft tissue dehiscences (PSTDs)



1.2.3 Prevalence and risk indicators of Peri-implant soft tissue dehiscences

Regarding its prevalence, a PSTD is not a rare finding. In a 2-year prospective study, Bengazi et al. (1996) reported a 57% incidence of PSTD ≥ 1 mm (on the facial or lingual sites) during the first 6 months. Interestingly, the authors found no further progression in the following months [55]. Among the factors that can lead to a mucosal recession, Lin et al. proposed that lack of or a minimal KM around implants may play a crucial role [10]. According to a systematic review by Chen & Buser, immediately placed implants are associated with a higher risk of facial PSTD (from 9 to 41%) [56], possibly due to the insufficient experience of the surgeon or (site specific) anatomical limitations [57, 58]. In this view, Evans & Chen also discovered a significantly greater apical shift of the soft tissue margin in patients with a thin tissue phenotype (i.e. biotype) [59]. Additionally, they also highlighted the importance of the position of the implant shoulder, which correlated with a 3 times greater risk of producing a PSTD if buccally placed, compared to a lingually positioned one [59].

The relatively high prevalence of a midfacial PSTD that can range up to 64% in immediate implants [60]. This could be attributed to many predisposing and precipitating factors including: a buccally positioned implant, an osseous dehiscence or fenestration at the buccal bone, a thin gingival phenotype, a lack of or a minimal KM, vigorous toothbrushing,

inflammation and an over-contoured prosthesis [45]. While some of these factors are also present in the case of gingival recessions around natural teeth [45], for a PSTD to occur around implants it is believed that among all the predisposing factors, the bucco-lingual positioning of the fixture is the most crucial causative factor [59, 61]. A recent cross-sectional study by Sanz-Martin et al. showed that implants with PSTDs had less KM width, higher bleeding on probing and plaque scores, higher first bone to implant contact at the buccal aspect and were more buccally positioned compared to implants without PSTDs [62]. In particular, implants buccally positioned in the CBCT's were 34 times more likely to belong to the case group. The authors observed that the presence of KM width of at least 2 mm, presence of adjacent natural teeth, cemented restorations and two-piece implants were protective factors for PSTDs [62]. Another recent study investigated factors associated with this condition, reporting an overall prevalence of PSTD in healthy implants of 12% [63].

While these investigations provided interesting findings with important clinical relevance, it must be kept in mind that PSTD was defined as the exposure of the prosthetic abutment or the implant neck [62, 63] and not as described in the recent classification of PSTD from Zucchelli et al. [43].

1.2.4 Treatment of Peri-implant soft tissue dehiscences: soft tissue grafting techniques and materials

Given the role that the soft tissue component can play on peri-implant health and esthetics, it is not surprising that clinicians have investigated different techniques to convert the peri-implant soft tissue phenotype and to treat PSTDs. Among these approaches, autogenous soft tissue grafts (either FGG or CTG) have been the first tissue sources that were explored due to the promising results shown around natural dentition [36, 64].

In particular, according to Zuhr et al., the introduction of CTG [65] and the increasing changeover from the FGG to a CTG presents the transition from traditional mucogingival surgery to periodontal plastic surgery [66]. While traditional mucogingival approaches were aimed primarily at increasing the KTW, the principal goal of modern periodontics should embrace the ultimate esthetic outcomes [66, 67]. There is extensive evidence that a CTG is the material of choice in treating gingival/mucosal recessions at teeth and implant sites [42, 68], for increasing soft tissue thickness [69], masking discolored roots or visible implant components [66], as well as interdental papilla reconstruction [70].

CTG-based approaches demonstrate the strongest potential of achieving complete root coverage, together with the highest esthetic results around natural dentition [24, 64, 67, 68]. While the FGG retains its original appearance of the palatal soft tissue at the recipient site [71] and may result in poor esthetic appearance and a scar tissue-like texture [66], the CTG is able to increase soft tissue volume and quality, as well as provide a harmonious gingival margin [66, 67]. The use of CTG for the treatment of PSTDs has been highly recommended, regardless of KM width or thickness [24, 44, 72]. In particular, it has been suggested that the harvesting technique may also affect the quality of the graft, being a CTG derived from de-epithelialization of a FGG mainly composed of lamina propria, while a CTG from conventional harvesting approaches (i.e., deep palate) are more rich in glandular and adipose tissue [66, 73-75]. This dissimilar nature of the graft renders a CTG from the superficial palate distinctively different from a CTG from the deep palate, with the CTG obtained from the de-epithelialization of an FGG firmer, more stable and

easier to manage [73, 74]. Furthermore, since a CTG can promote the keratinization of the overlying epithelium [76], it has been speculated that the adipose and glandular tissue of the graft may act as barriers to the plasmatic diffusion and vascularization during the first phase of healing, and also impair their ability to induce epithelial keratinization [77, 78].

Nevertheless, patient morbidity and the need for a second surgical site [79, 80] has led clinicians to explore alternative grafts, such as acellular dermal matrix or xenogeneic collagen matrix [35, 36]. CTG substitutes are often used for increasing tissue thickness and minimizing the post-operative mucosal recession during immediate implant placement [81] or at the time of implant uncovering [28, 82]. These scaffold-based extracellular materials are devoid of cells and usually cellular signaling molecules and therefore they are aimed at promoting MT gain and not KM neogenesis. Based on their origin, these materials can be classified as: i) decellularized human dermis, ii) bilayer collagen matrix, iii) volume-stable collagen matrix and iv) xenogeneic acellular dermal matrix. Most of the evidence available in the literature regarding these graft substitutes is focusing on soft tissue augmentation in natural dentition.

1.2.5 Treatment of Peri-implant soft tissue dehiscences: evidence from previous studies

A large body of evidence supports the efficacy of periodontal plastic surgery for treating gingival recessions is available in the literature. However, when the same surgical approaches with the same biologic principles have been applied to recession defects at implant sites (PSTDs), the clinical outcomes were not as satisfactory as around teeth.
Several techniques, such as the coronally advanced flap (CAF) [83, 84], the tunnel (TUN) [85], the VISTA technique [86], free gingival grafts [87], guided bone regeneration procedures [88], resubmergence technique [89, 90] or a surgical-prosthetic approach in combination with an envelope coronally advanced flap (eCAF) [72], have been described for treating PSTDs around implants.

It should be highlighted that most these studies are either case reports or case series and therefore, this aspect should be taken into consideration when evaluating the generalizability of their outcomes (Supplementary Table 1 of the Appendix). In an underpowered clinical trial on 13 patients, Anderson et al. evaluated the effect of coronally advanced flap with the traditional flap design involving two vertical releasing incisions (CAF) with the addition of CTG (harvested from the deep palate) or acellular dermal matrix. A mean PSTD coverage of 40% and 28% was found for CAF + CTG and CAF + acellular dermal matrix, respectively. The limited amount of recession coverage was shown by patients' self-reported esthetics that did not change after the treatment [83]. In a pilot study, Burkhardt et al. included ten patients presenting isolated PSTDs with unrestored contralateral tooth for comparison [84]. The PSTDs were treated with CAF and a subepithelial CTG with the flap that was coronally repositioned with an overcompensation of 1.2 mm, on average. The authors observed a shrinkage of the soft tissue over time with a mean PSTD coverage of 75% at 1 month and 70% at 3 months. The final mean PSTD was 66% at the 6 months follow-up, with none of the sites showing complete PSTD coverage. The authors suggested that the lack of a periodontal ligament and the overall reduced blood supply of the peri-implant tissues may be one of the factors that negatively affected the outcomes [84].

Zucchelli and coworkers reported higher outcomes using a prosthetic-surgical approach, involving the removal of the crown and the surgical procedure performed in the presence of the abutment only, with the new definitive crown that was delivered only several months following the soft tissue augmentation procedure [72]. Nevertheless, it has to be considered that the removal of the implant-supported crown is often not feasible due to patient's concern and financial restriction.

In addition, it may be reasonable to assume that the variability in the obtained outcomes, such as a vast difference in mean PSTD coverage (from 40-66% in some trial [83, 84], to 90-96% in others [52, 72]) does not only depend on the surgical approach, but also on the case selection. Thus, it is crucial to pre-surgically differentiate the types of PSTDs using the previously mentioned classification.

2. AIMS OF THE STUDY

2.1 Aim of the study 1

The aim of the study was to evaluate the prevalence of PSTD and some clinical and ultrasonographic risk indicators for this condition.

2.2 Aim of the study 2

The aim of the clinical trial was to compare the clinical, volumetric, and ultrasonographic outcomes of PSTDs treated with CTG either with CAF or TUN.

3. MATERIALS AND METHODS

3.1 A clinical and ultrasonographic study cross-sectional study assessing the prevalence and risk indicators for peri-implant soft tissue dehiscences (Study 1)

3.1.1 Study design and Ethical considerations

The study was approved by the University of Michigan Medical School Institutional Review Board (IRBMED) (HUM00176741), in accordance with the Helsinki Declaration of 1975, as revised in 2013. An informed consent was obtained from all individuals who had participated in the study. The present cross-sectional study follows the STROBE statement [91].

3.1.2 Inclusion/exclusion criteria

Subjects with one or more healthy dental implants in the esthetic area (from the right first premolar to the left first premolar) were identified and recruited from a population attending the Graduate Periodontics clinic at the Department of Periodontics and Oral Medicine, School of Dentistry, University of Michigan, Ann Arbor, USA between February 2020 and June 2021. The inclusion criteria were: 1) systemically and periodontally healthy subjects, 2) having at least one anterior dental implant with two adjacent natural teeth and/or dental implants, 3) dental implant(s) diagnosed as healthy ("absence of erythema, bleeding on probing, swelling and suppuration"[92]), 4) dental implants rehabilitated with a single implant-supported crown, 5) loading time of at least 24 months [62] , 6) presence of the homologous contralateral natural tooth, 7) available information regarding implant characteristics and 8) patients willing to provide an informed consent and attend the study. Exclusion criteria included: 1) Multiple adjacent dental implants with PSTDs, 2) implants in the second premolar or molar region, 3) one or two adjacent edentulous area, 4) implant(s) restored with three (or more)-unit fixed bridges, single crown with cantilever or removable prosthesis, 5) current diagnosis of active periodontitis, 6) any confirmed peri-implant disease [92], 7) documented history of peri-implantitis or previous surgical procedures at the implant site, 8) previous soft tissue graft at the implant site and 9) missing information on the implant characteristics. The patient recruitment process, clinical assessment and ultrasonographic examination were performed by two calibrated study team members (L.T. and S.B.) following a standardized protocol as previously described [93].

3.1.3 Data Collection and Clinical measurements

At the time of the visit, patient demographics and implant characteristics were obtained, as well as the following parameters by a single examiner:

- Presence or absence of PSTD, defined as the apical shift of the mucosal margin compared to the gingival margin of the homologous contralateral natural tooth [43]. In case of a PSTD, the class (I, II or III/IV) and subclass (a, b or c) were also identified [43]. Since the implant-supported crown was not removed in the present study, implants with a PSTD characterized by a crown profile located outside (more facial to) an imaginary curve line connecting the profile of the adjacent teeth at the level of the mucosal margin were considered as class III/IV.
- Presence or absence of an implant-supported crown longer than the clinical crown of the homologous contralateral natural tooth
- Presence or absence of the exposure of the abutment and/or implant fixture to the oral cavity
- Presence of adjacent (mesial/distal) implants
- Probing pocket depth (PD) using a periodontal probe (PCP UNC 15, Hu-Friedy, Chicago, IL, USA)
- KMW, defined as the vertical distance between the mucogingival junction and the mucosal margin in the mid-facial region, and measured with a periodontal probe (PCP UNC 15, Hu-Friedy, Chicago, IL, USA).

3.1.4 Ultrasonographic image acquisition and measurements

The ultrasound equipment setup and the scanning procedures have been previously described in detail [94-97]. Briefly, a commercially available ultrasound imaging device (ZS3, Mindray, Mountain View, CA, USA) was coupled with a 24 MHz (64 μm axial image resolution) and miniature-sized (approximately 30 mm long, x 18 mm wide x 12 mm thick) probe prototype (L30-8) to generate ultrasound images (pixel size 0.05 mm) (Figure 2).

Figure 2. Ultrasound machine, transducer, and its intraoral application.

Single image frames ("still images") at the mid-facial aspect of the implant(s) of interest were saved in "B-mode" in the Digital Imaging and Communications in Medicine (DICOM) format. "B-mode" generates 2D grey-scale images in which brightness is the result of the returned echo signal and its strength, which depends on the acoustical properties of the implant components and the peri-implant soft and hard structures. The US probe was oriented perpendicular to the occlusal plane and parallel to the long axis of the implant at its midfacial aspect [95, 96].

The following measurements were computed using a commercially available software package (HorosTM, version 3.3.6, Horos Project), as previously described [93, 95-98] and were carried out by a single experienced examiner, who has been calibrated in previous trials ($k \geq 0.87$):

- MT: horizontal thickness of the peri-implant soft tissue, calculated as the distance between the soft tissue margin and the abutment/implant fixture/buccal bone on a line parallel to the long axis of the implant body in the mid-facial scan. MT was measured at 1 and 3 mm (MT1 and MT3, respectively) from the soft tissue margin.
- Peri-implant buccal bone distance (BBD): Distance between the implant platform and the peri-implant bone crest evaluated on a line parallel to the long axis of the implant body in the mid-facial scan.
- Peri-implant buccal bone thickness (BBT): evaluated 0.5 mm apical to the bone crest as the distance between the peri-implant crestal bone and a line parallel to the long axis of the implant body in the mid-facial scan

Figures 3 and 4 depict dental implants and their midfacial ultrasonographic scans, highlighting the above-mentioned parameters that were investigated.

Figure 3. Clinical and ultrasonographic presentation of an implant without PSTD (A, A' and A''), PSTD with longer crown (B, B' and B''), PSTD with an adequate crown length and abutment exposed (C, C' and C''), PSTD with a crown longer than the clinical crown of the homologous tooth and with abutment exposed (D, D' and D''). The midfacial ultrasonographic scans show the soft tissue (ST) highlighted in green, the implant-supported crown (Cr), the abutment (Ab), the implant threads above the bone (IT) and the peri-implant crestal bone (CB) and the ultrasonographic outcomes of interest (BBT, BBD, MT1 and MT3) [99].

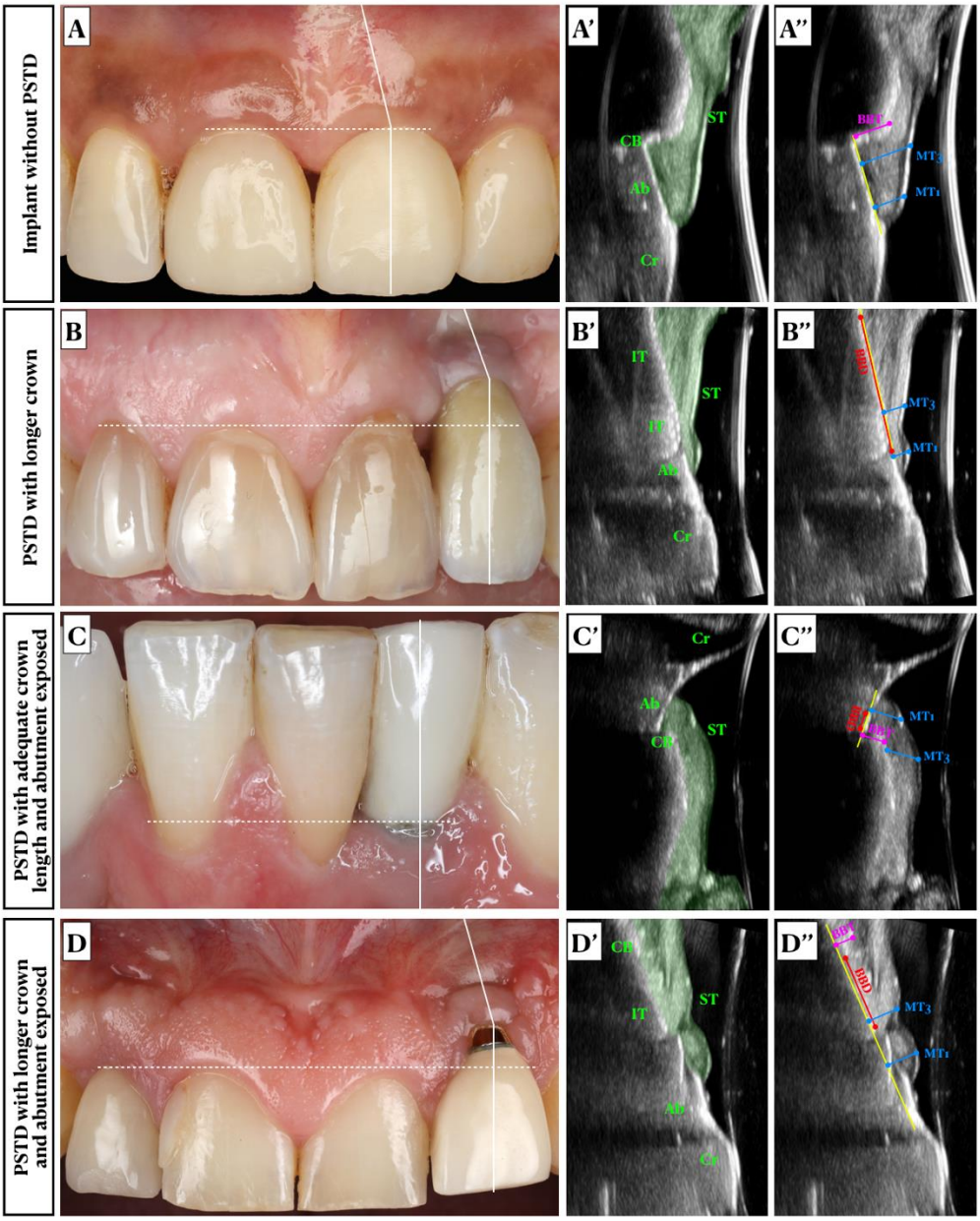
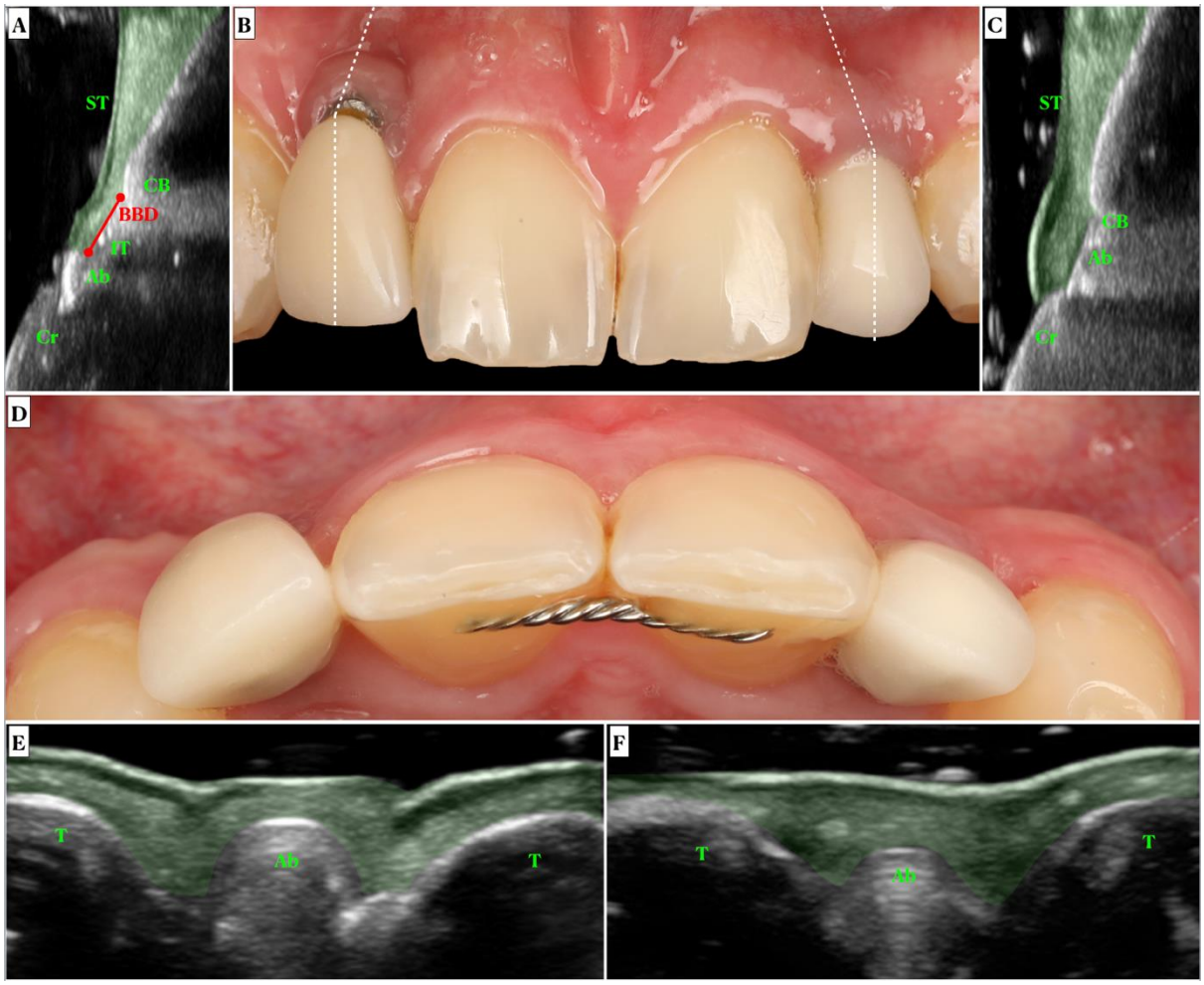


Figure 4. Subject with two dental implants in the lateral incisor position (A-F). The left implant shows a soft tissue dehiscence with the abutment exposed, while the implant on the right does not display a soft tissue dehiscence. A) Midfacial ultrasonographic scan of the implant with peri-implant soft tissue dehiscence, where the soft tissue component (ST) is highlighted in green. The implant-supported crown (Cr), the abutment (Ab), the implant threads above the bone crest (IT) and the peri-implant crestal bone (CB) are displayed. Note that the implant has the abutment exposed to the oral cavity and several threads above the CB. Thin mucosa (MT1= 0.39 mm and MT3= 0.89 mm) and buccal bone distance (distance from the implant platform to the CB, BBD= 2.35 mm, highlighted in red) may have contribute to the clinical manifestation of the PSTD. The implant on the left side displays a thicker mucosa (MT1 = 1.39 mm and MT3 = 1.84 mm) without bone loss (BBD = 0) (C). D) Occlusal view of the two implants where it is possible to appreciate that the implant with PSTD was placed more buccally (PSTD class III) than the implant without PSTD. E-F) Transverse ultrasonographic scan showing the soft and hard structures of the right and left implant, respectively. The adjacent teeth (T) are also highlighted [99].



3.1.5 Data Collection and Statistical analysis

All clinical, ultrasonographic and demographic data were entered into a single prefabricated spreadsheet. Descriptive statistics were used to illustrate the overall clinical and ultrasonographic-related parameters with means and standard deviations (SD) for continuous measures, among implants with and without PSTDs.

To test for statistically significant relationships among the collected variables of interest to the primary outcome PSTD (Yes/No), logistic regression models were fit with generalized estimating equation (GEE), that accounted for repeated measures (more than 1 implant per patient) across observed sample.

A stepwise regression approach was utilized to univariately introduce the variables of interest for testing their predictive values and kept for multi-variate modeling if obtained a p of < 0.05.

For significant predictors, the final coefficients from the multi-variate model were recorded, and exponentiated to produce odds ratios (OR). Confidence intervals (CI) were produced and a p value of 0.05 was set for statistical significance. The analyses were performed in software (Rstudio Version 1.1.383, Rstudio, Inc., Boston, MA, USA).

3.2 Coronally Advanced flap vs Tunnel technique for the treatment of peri-implant soft tissue dehiscences: A randomized, controlled, clinical trial (Study 2)

3.2.1 Study design and trial registration

The present study was designed as a double-blind, parallel arm, randomized, controlled clinical trial on the treatment of PSTDs to compare two procedures in combination with CTG: the Coronally Advanced Flap (CAF) and the Tunnel technique (TUN). The trial was registered at ClinicalTrial.gov (NCT03498911) and follows the CONSORT statement (http://www.consort-statement.org/) (Supplementary Figure 1 of the Appendix). The study protocol was approved by the Institutional Review Board of the University of Michigan Medical School (HUM00140205) and is in accordance with the Declaration of Helsinki of 1975, revised in Fortaleza in 2013.

All participants were informed and understood the objectives and the details of the study and signed a written consent form.

3.2.2 Inclusion/exclusion criteria

Subjects presenting with PSTDs in non-molar sites were screened at the Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry (Ann Arbor, USA) between July 2018 and September 2020.

Patients satisfying the following inclusion criteria were recruited: i) Age $\geq$ 18 years, ii) Periodontally and systemically healthy, iii) Full-mouth plaque score and full-mouth bleeding score $\leq$ 20%, iv) Dental implants with isolated Class II PSTDs, subclass a or b [43], located in a non-molar site and with two adjacent natural teeth, v) Osseointegrated and functionally loaded dental implants and vi) No history of previous PSTD treatment at the implant site.

Exclusion criteria were: i) Contraindications for surgery, ii) Class I, III or IV PSTDs or subclass c PSTDs [43], iii) Multiple adjacent implants with PSTDs, iv) PSTDs with implant-supported crown margin located $\geq$ 3 mm apical than the gingival margin of the homologous contralateral tooth, v)

Diagnosis of peri-implantitis [53], vi) Previous mucogingival surgery around the implant within the past six months and vii) Smoking more than 10 cigarettes a day

3.2.3 Intervention

Eligible patients received a session of dental prophylaxis, including oral hygiene instruction aimed at eliminating possible traumatic toothbrushing habits at least 1 month before the surgery. The surgical treatment was not scheduled if the patient could not demonstrate an adequate standard of plaque control. All surgical procedures were performed at the Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry (Ann Arbor, USA) by a single clinician (L.T.).

Coronally advanced flap with connective tissue graft (CAF group)

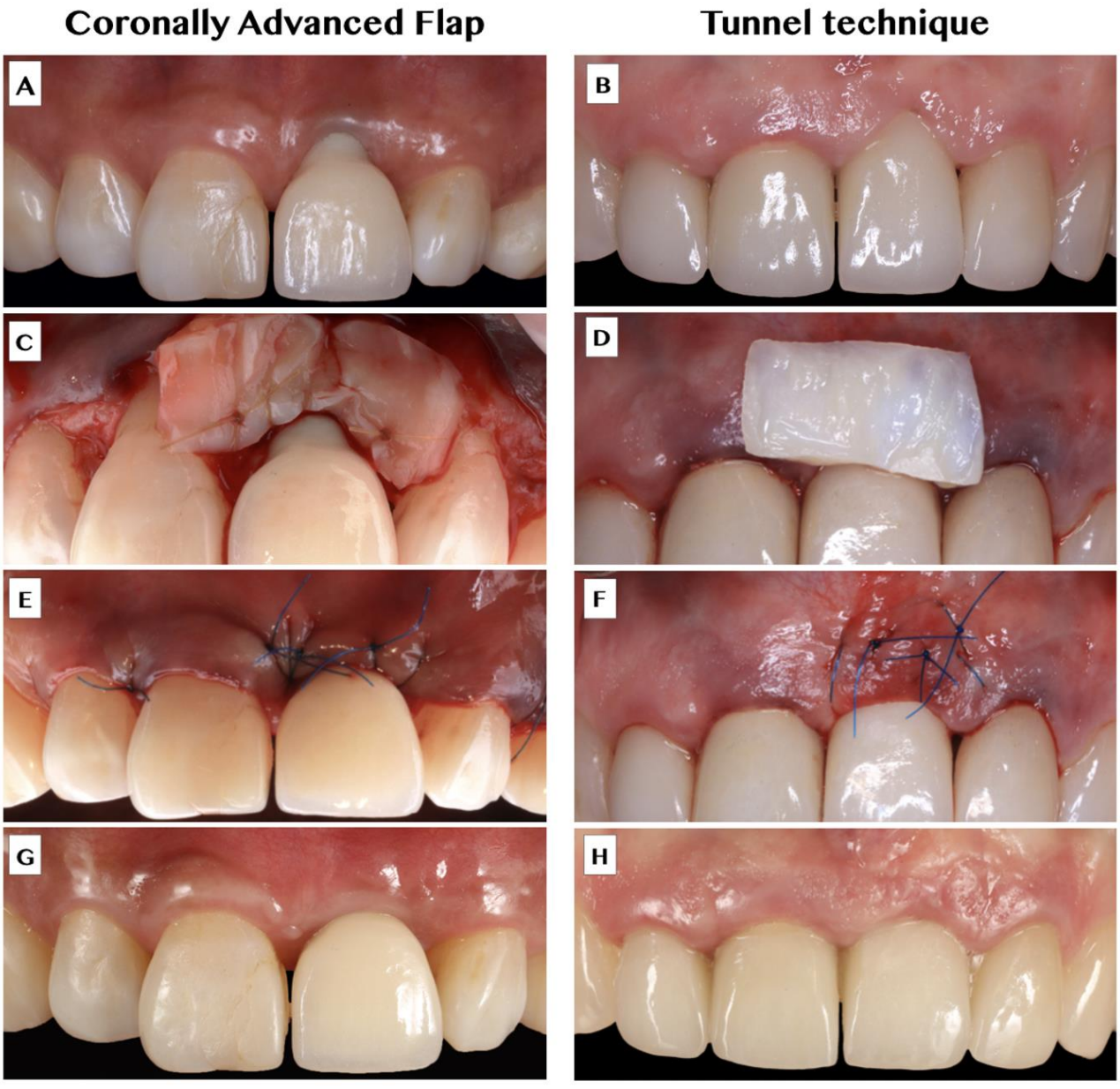
A CAF, as previously described by Zucchelli et al. [100], was performed to treat the PSTDs allocated to the CAF group. Briefly, oblique submarginal incisions were performed with mini-blades (Mini Blade #67, Salvin Dental Specialties, Charlotte, USA) at the level of the interdental soft tissue of the implant with PSTD. The papilla of the adjacent (mesial and distal) teeth were also incised and included in the CAF (Figure 5). The flap was raised with a split approach at the level of the interproximal soft tissue and also at the midfacial aspect of the implant - when possible - aiming at maintaining some connective tissue attached to the implant fixture surface not covered by bone. The elevation of the midfacial area of the adjacent teeth was performed full-thickness. Then, the elevation of the flap proceeded split thickness to release the tension. The anatomical papillae were de-epithelialized with a mini-blades (Mini Blade #67, Salvin Dental Specialties, Charlotte, USA). Implant/abutment surface decontamination was performed (using 0.12% chlorhexidine solution and titanium brushes [Salvin Dental Specialties, Charlotte, USA]) only if part of the implant fixture or abutment was already exposed to the oral cavity prior to the intervention. The soft tissue left on the implant surface and buccal bone with the split-thickness flap elevation was preserved. A free gingival graft was harvested from the palate, approximately 1-2 mm below the gingival margin from the first and second premolars. The width and the height of the graft depended on the implant site, while a thickness of 1.5-2 mm was aimed [74]. The graft was then extraorally de-epithelialized to obtain a CTG [101]. The graft was positioned over the implant surface and stabilized with simple interrupted sutures (5-0 and 6-0 chromic gut, Ethicon, Johnson & Johnson, USA), using the de-epithelialized anatomical papillae and the periosteum as anchorage. The flap was coronally advanced and sutured 1-2 mm above the ideal position of the soft tissue with sling sutures (6-0 polypropylene, Ethicon, Johnson & Johnson, USA) (Figure 5).

Tunnel technique with connective tissue graft (TUN group)

A modified TUN as previously described by Aroca et al. and Zuhr et al. [102, 103] was performed in implants with PSTD allocated to the TUN group. A rounded mini-blade (Mini Blade #69, Salvin Dental Specialties, Charlotte, USA) was utilized for the intrasulcular incisions performed around the PSTD and the sulcus of the adjacent teeth. A tunnel flap was executed with tunneling knifes (tunneling knife #1 and #2, American Dental Systems, Vaterstetten, Germany) and extended toward the apical area and the adjacent teeth taking care not to lacerate the soft tissue. The papillae were preserved and detached from the underneath interproximal bone using a papilla elevator (Micro papilla elevator, American Dental Systems, Vaterstetten, Germany). The flap was considered tension free when it could be easily coronally advanced 2 mm more than the ideal position of the peri-implant soft tissue margin. A CTG was harvested from the palate and de-epithelialized as described for the CAF group. The CTG was introduced underneath the tunnel flap and stabilized with interrupted and

sling sutures to the flap (6-0 polypropylene, Ethicon, Johnson & Johnson, USA). Afterward, the flap was stabilized 1-2 mm coronal to the ideal position of the peri-implant soft tissue margin with sling sutures. If further coronally advancement was needed, modifies sling sutures around composite stops placed to close the interproximal spaces were also performed [102, 103] (Figure 5).

Figure 5. Surgical intervention in a patient allocated to the CAF + CTG or to the TUN + CTG group. A-B) Baseline showing isolated PSTDs subclasses. C) Stabilization of the CTG to the periosteum and to the surgical papillae. D) CTG prior to its insertion underneath the tunnel flap. E-F) Flap closure. G-H) 6-month outcomes.



A collagen dressing (Collatape, Zimmer Biomet, USA) was applied to the palatal donor site and secured with simple and cross sutures (5/0 Vicryl, Ethicon, Johnson & Johnson, USA). A layer of cyanoacrylate tissue glue (PeriAcryl 90 HV, Glustitch, Delta, Canada) was also applied over the collagen sponge and around the edge of the palatal wound [104, 105].

3.2.4 Post-operative instruction and medication regimen

Patients were instructed to avoid mechanical trauma and brushing at the surgical site for two weeks and to intermittently apply an ice pack for the first 24 hours. Patients were prescribed Ibuprofen 600 mg every 4-6 hours for the first 3 days, followed by its prescription as needed for pain/discomfort and Chlorhexidine mouth rinse 0.12 % twice daily for one minute for 14 days. Smokers were reminded to quit smoking during the first two weeks. The sutures were removed two weeks after the surgical procedure. Patients were instructed to resume mechanical tooth brushing using an extra-soft bristle toothbrush at the operated area. At the 1-month appointment, patients were given a soft bristle toothbrush to replace the extra-soft bristle one, and oral hygiene instructions were reinforced.

3.2.5 Study outcomes

The primary outcome of the study was to compare the mean peri-implant soft tissue dehiscence coverage (mean PSTD coverage) at 6 months between the two groups.

The secondary outcomes that were analyzed and compared within the two groups included: i) frequency of complete peri-implant soft tissue dehiscence coverage; ii) Keratinized mucosa width gain; iii) Attached mucosa width gain; iv) Mucosal thickness gain; v) Soft tissue volume change over time, using optical intraoral scanning; vi) Ultrasonographic peri-implant phenotype (in terms of mucosal thickness, buccal bone position, and supracrestal tissue height) changes; vii) Professional esthetic evaluation, using the Implant soft tissue Dehiscence coverage Esthetic Score (IDES) (Supplementary Table 2 of the Appendix) [106].

3.2.6 Clinical measures

The following clinical measurements were performed by a single masked calibrated examiner at baseline, 3 months, and 6 months after surgery at each experiment site.

PSTD depth and KMW were measured with a periodontal probe (PCP UNC 15, Hu-Friedy, Chicago, USA) and rounded up to the nearest 0.5 millimeter.

The following clinical measurements were collected at baseline, 3 months, and 6 months after surgery at each experiment site:

- Peri-implant soft tissue dehiscence (PSTD) depth: corono-apical distance between the peri-implant soft tissue margin and the ideal soft tissue margin, defined based on the level of the homologous contralateral unrestored tooth [43, 72];
- Pocket depth (PD): measured from the soft tissue margin to the bottom of the peri-implant sulcus;
- Clinical attachment level (CAL): obtained by adding PD to PSTD depth;
- Keratinized mucosa width (KMW): corono-apical width/height measured from the soft tissue margin to the mucogingival junction and identified using Lugol staining [72];

- Attached mucosa width (AMW): obtaining by calculating the difference between KMW and PD;
- Mucosal thickness (MT): measured 1.5 mm apical to the soft tissue margin using a short injection needle for anesthesia and a silicon disk stop, which was then fixed with a few drops of cyanoacrylate as described by Zucchelli and coworkers [74] [100]. After needle removal, the distance between the tip of the needle and the disk stop was measured with a digital caliper with 0.01 mm accuracy.

PSTD depth, PD and KMW were measured with a periodontal probe (PCP UNC 15, Hu-Friedy, Chicago, USA) and rounded up to the nearest 0.5 millimeter.

3.2.7 STL file acquisition and volumetric outcomes assessment

An intraoral optical scanner (Trios, 3Shape, Denmark) was utilized to generate digital models that were saved as STL files and imported in an image analysis software (GOM Inspect, GOM, Germany). A blinded and pre-calibrated examiner with experience in the 3D volumetric analysis performed all the measurements. A semi-automated alignment, based on the selection of reproducible points on the digital models and on a best-fit algorithm, was used to superimpose the STL files [107]. Each time point (1, 3 and 6 months) was superimposed to baseline, which was used as the reference. The region of interest (ROI) was defined as previously stated [108]. The volumetric outcomes of interest were volume change in mm^3 (Vol) and the mean distance between the surface/ mean thickness of the reconstructed volume in mm (ΔD) [108, 109].

3.2.8 Ultrasonographic protocol and data acquisition

The ultrasound (US) equipment setup and the scanning protocols have been described for the study 1 (see paragraph 3.1.4). The following outcomes of interest were assessed on the midfacial US scan at baseline, 1 month and 6 months as previously described in detail [95, 110, 111], utilizing a public-domain software package (Horos™, version 3.3.6, Horos Project): i) Mucosal thickness, evaluated 1, 3 and 5 mm apical to the soft tissue margin of the implant (UMT 1, UMT 3, and UMT 5, respectively); ii) Distance between the crown margin and the soft tissue margin (CM-STM); iii) Distance between the implant shoulder and the bone crest (ultrasonographic buccal bone dehiscence [BBD]); iv) Distance between the crown margin and the crestal bone (CM-CB); v) Supracrestal tissue height (STH), defined as the distance from the crestal bone to the soft tissue margin [5]. In addition, the buccal bone dehiscence measured clinically in CAF-treated sites (BD) was compared to the preoperative measurements of buccal bone dehiscence obtained from the ultrasound scans (BBD) (Supplementary Figure 2 of the Appendix).

3.2.9 Ultrasonographic Power Doppler

The preliminary pilot analysis of blood flow changes at grafted implant sites and palatal donor site was performed on a sample of 5 patients over a period of 12 months.

Ultrasound scans were obtained as described above. In addition, scans were also acquired as “cine loops” videos in the “color” modality. “Color flow” is an imaging mode in

which the B-mode display is overlaid with additional color pixels that represent detected blood flow. In this case B-mode provides an anatomical reference for the physical location of the detected blood flow [95]. "Color flow", also known as "color Doppler", detects phase-changes in the received ultrasound signal. The displayed color velocity (CV) is the projection of the actual velocity onto the ultrasound beam, which mathematically equals the multiplication of the true velocity by the cosine of the angle to the US beam. CV visualizes the speed at which blood flows within the lumens in the field of view. "Color power" (CP) is an imaging mode that is also based on detecting phase-change of the received ultrasound signal that displays the integrated power of the received ultrasound signal. This power is displayed in a single-hue red color. CP visualizes the *amount* of blood flowing within the lumens in the field of view.

The areas of interest at the implant site were: i) midfacial, ii) mesial (at the line angle between the crown and the mesial papilla), iii) distal (at the line angle between the crown and the distal papilla) and iv) transverse scan at 3 mm from the mucosal margin level [95]. For the midfacial, mesial and distal scans, the US probe was oriented parallel to the long axis of the implant and perpendicular to the occlusal plane, while for the transverse scan the probe was oriented parallel to the occlusal plane. The areas of interest at the palatal donor site were: 3-, 5-, and 8-mm reference points apical to the gingival margin of the first and second premolars, and the greater palatine foramen (GPF) area, which was identified by palpation at the junction between the horizontal plate of the maxilla and alveolar ridge at the 3rd molar location [79, 112]. For each area of interest at the implant and palatal site, B-mode, CV and CP scans were performed and saved as still images (for the B-mode) and cine loop videos (for CV and CP modes) at the baseline, 1 week, 1 month, 6-months and 12-months.

Speed-weighted and power-weighted color pixel densities were computed by a calibrated examiner with expertise in ultrasound imaging and image/signal processing using custom scripts for Matlab (The Mathworks, Natick, MA). All data were processed using the same scripts to improve scientific rigor and eliminate bias. CV_m and CP_m were obtained as an average of CV/CP_w across at least 5 the cardiac cycles (6 second cine clips at minimum 20 Hz frame rate for cardiac averaging). The variation in percentages compared to baseline was computed and descriptive statistics were used to present the gathered data as means ± standard deviations (SD).

3.2.10 Sample size and statistical analysis

The study was powered to detect a minimum clinically significant difference in soft tissue dehiscence coverage of 0.5 mm using $\alpha = 0.05$, a power (1- β) of 80%, and a hypothesized within-group sigma of 0.446 mm [83]. Considering possible dropouts, the number of patients were increased by 15% for each arm. On the basis of these data, the minimum number of patients needed to be enrolled in this study was in 28 totals, with 14 for the CAF + CTG group, and 14 for the TUN + CTG group.

All observed data and the collected variables were entered into a prefabricated spread sheet, based on the patients' original IDs, without knowledge of their group allocation. Means and standard deviations (SD) were calculated for continuous measures. Complete soft tissue dehiscence coverage was calculated as the percentage of sites that achieved a complete at 6 months and expressed as a binary outcome. T-test was utilized to compared baseline and the

6-month outcomes between the two interventions. Linear mixed-effects and logistic regression models were used to assess statistical changes in PSTD depth between different time points and differences between the CAF and TUN groups. The randomization, as to which among the two groups (1 or 2) had served as the TUN sites was revealed at the end of the analysis by the study coordinator. All analyses were performed by an individual author with experience in biostatistical analyses, who had not taken part in the clinical measurements with a specified software (RStudio, Version 1.3.959).

4. RESULTS

4.1 A clinical and ultrasonographic study cross-sectional study assessing the prevalence and risk indicators for peri-implant soft tissue dehiscences (Study 1)

4.1.1 Experimental population and dental implants characteristics

One-hundred and fifty-three subjects (80 males and 73 females, with a mean age of 59.5 ± 15.6 years) with a total of 176 dental implants were included in the present study (Supplementary Table 3 of the Appendix). Among them, 54.2% patients had at least one implant with a PSTD. On an implant-level, 100 dental implants (56.8%) displayed a PSTD and 76 (43.2%) did not. Eighty-four percent (84%) of the implants with a PSTD showed a crown longer than the clinical crown of the homologous tooth, while the exposure of the abutment or implant fixture to the oral cavity was present in 74% of sites with a PSTD. The most frequent type of PSTD was the one characterized by having both an implant-supported crown longer than the clinical crown of the homologous tooth and a visible abutment/implant fixture exposed to the oral cavity (58% of the PSTD cases). Most of the implants with PSTD were diagnosed with class III/IV (58%), while 39% and 3% of cases were classified as PSTD class II and class I, respectively. The most frequent PSTD subclasses were subclass c and subclass b (52% and 40%, respectively) (Supplementary Table 4 of the Appendix).

The mean time in function of the implants with PSTD was 9.3 ± 4.5 years, while for implants without PSTD was 4.9 ± 1.6 years. Implants with PSTD had an adjacent dental implant (without PSTD) in 54% of cases, while implants without PSTD had an adjacent implant (without PSTD) in 5.3% of cases. The mean PD was 2.6 ± 0.6 mm and 2.6 ± 0.8 mm in implants with and without PSTD, respectively, while the mean KM width was 2.2 ± 1.7 mm and 4.5 ± 1.7 mm in implants with and without PSTD, respectively.

4.1.2 Ultrasonographic outcomes

Descriptive summaries of the measured clinical variables are reported in the Supplementary Table 3 of the Appendix. The measurements of MT at the midfacial ultrasonographic scans tended to be higher at sites without PSTD compared to implants with PSTD (mean MT1 of 1.51 ± 0.58 mm vs 0.65 ± 0.36 mm and mean MT3 of 2.05 ± 0.79 mm vs 1.35 ± 0.56mm, respectively). The average BBD was also higher at implants with a PSTD (3.25 ± 2.07 mm for implants with a PSTD versus 1.73 ± 1.20 mm for implants without), while a mean BBT of 0.91 ± 0.43 mm, and 1.48 ± 0.66 mm was observed for implants with and without PSTD, respectively.

4.1.3 Risk indicators for the presence of a peri-implant soft tissue deficiency (PSTD)

Table 1 displays the results of the logistic regression models for the outcome of PSTD. The uni-variate analysis showed that the variables of presence of an adjacent implant (OR 14.4 (95% CI [3.22, 64.8]), p<0.001), implants' time in function (OR 1.73 (95% CI [1.47, 2.03]), p<0.001), KMW (OR 0.49 (95% CI [0.38, 0.63]), p<0.001), MT1 (OR 0.08 (95% CI [0.04, 0.17]), p<0.001), MT3 (OR 0.37 (95% CI [0.22, 0.63]), p<0.001), BBD (OR 1.86 (95% CI

[1.35, 2.56]), p<0.001), and BBT (OR 0.09 (95% CI [0.02, 0.37]), p=0.001) were significantly related to higher odds of the presence of a PSTD.

The multi-variate analysis confirmed that the presence of an adjacent implant increases the odds of having a PSTD by a factor of approximately 11 (OR 10.9 (95% CI [2.98, 40.2]), p<0.001), as well as the time (in years) of the implants in function (OR 1.4 (95% CI [0.71, 2.73]), p=0.001). Additionally, the model showed an inverse correlation between MT both at 1 mm (OR 0.11 (95% CI [0.04, 0.24]), p<0.001), and 3 mm (OR 0.34 (95% CI [0.14, 0.82]), p=0.01) from the mucosal margin, and the amount of KMW (OR 0.73 (95% CI [0.55, 0.97]), p<0.001), with the presence of PSTD among the population cohort. Relative to the peri-implant buccal bone, BBD also was significantly associated with the presence of a PSTD (OR 1.41 (95% CI [1.02, 1.95]), p<0.001).

Table 1. Uni- and multi-variate results of the logistic regression models assessing the correlation of PSTDs to the observed variables.

Variable	**Univariate analysis**			**Multivariate analysis**		
	OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
Gender (Male)	0.55	0.28, 1.1	0.09			
Age	0.98	0.94, 1.03	0.11			
Smoking	2.22	0.76, 6.51	0.14			
Presence of Adjacent implant	14.4	3.22, 64.8	<0.001	10.9	2.98, 40.2	<0.001
Years in function (time since installment of final prosthesis)	1.73	1.47, 2.03	<0.001	1.4	0.71, 2.73	0.001
KMW (mm)	0.49	0.38, 0.63	<0.001	0.73	0.55, 0.97	0.03
MT1 (mm)	0.08	0.04, 0.17	<0.001	0.11	0.04, 0.24	<0.001
MT3 (mm)	0.37	0.22, 0.63	<0.001	0.34	0.14, 0.82	0.01
BBD (mm)	1.86	1.35, 2.56	<0.001	1.41	1.02, 1.95	0.02
BBT (mm)	0.09	0.02, 0.37	0.001			

Legend. BBD: Buccal bone distance. BBT: buccal bone thickness. KMW: keratinized mucosa width. MT1: mucosal thickness evaluated 1 mm below the mucosal margin. MT3: mucosal thickness evaluated 3 mm below the mucosal margin.
OR: odds ratio.
CI: confidence intervals.

4.2 Materials and Methods Coronally Advanced flap vs Tunnel technique for the treatment of peri-implant soft tissue dehiscences: A randomized, controlled, clinical trial (Study 2)

4.2.1 Participant flow, baseline data and numbers analyzed

96 subjects were assessed for eligibility; among them, 63 did not meet the inclusion criteria and 2 declined to participate due to not being able to comply with all the recall appointments. Therefore, twenty-eight subjects (mean age 47.0 ± 12.1 years, 14 females, 12 males), 14 per group, each contributing with one experimental site only, were randomized and received the allocated interventions. All subjects completed the follow-up visits and complied with the study recall appointments. Patients' characteristics at baseline are depicted in the Supplementary Table 5 of the Appendix. Seven PSTDs allocated to the CAF group were classified as subclass a, while 6 PSTDs in the TUN group were judged as subclass a. All the implants that received PSTD treatment were bone level implants. No significant differences were observed between the two groups in terms of baseline clinical parameters ($p>0.05$). Similarly, the dimension of the CTG within the two groups were not statistically different (Supplementary Table 6 of the Appendix). The average surgical time was 82 ± 8 min and 80 ± 5 min for the CAF and TUN groups, respectively ($p>0.05$).

4.2.2 Clinical outcomes

The primary endpoint of the study was mean PSTD coverage at 6 months, that was significantly greater in the CAF group compared to the TUN group (87.85% vs 64.40%, $p=0.04$). Sites treated with CAF obtained also higher complete PSTD coverage (64.3% vs 42.9%, $p>0.05$), although this result was not statistically significant.

A significantly greater KMW gain (1.64 mm vs 0.82 mm, on average, $p=0.03$) and AMW gain (1.14 mm vs 0.36 mm, $p=0.03$) were observed at implants allocated to CAF compared to TUN, respectively. The mean MT gain, from baseline to the 6-month follow-up, was 1.44 mm and 0.99 mm, in the CAF and TUN group, respectively ($p=0.02$). Table 2 depicts the clinical outcomes at baseline and 6 months.

Linear regression analysis demonstrated that treatment approach ($p=0.042$) and PSTD subclass ($p=0.045$) were significantly associated with mean PSTD coverage. In other words, higher mean PSTD coverage should be expected for CAF and in presence of PSTDs subclass a. Age, sex, arch, baseline KMW and baseline MT were not associated with final mean PSTD coverage ($p>0.05$).

Table 2. Clinical outcomes at baseline and 6 months.

Outcome measure	CAF + CTG	TUN + CTG
Baseline		
PSTD depth (mean ± SD) (mm)	2.46 ± 0.87	2.36 ± 0.46
PD (mean ± SD) (mm)	2.14 ± 0.41	2.04 ± 0.46
CAL (mean ± SD) (mm)	4.61 ± 1.06	4.39 ± 0.81
KMW (mean ± SD) (mm)	1.96 ± 1.35	1.79 ± 0.99
AM (mean ± SD) (mm)	0.43 ± 0.81	0.29 ± 0.38
MT (mean ± SD) (mm)	1.18 ± 0.40	1.42 ± 0.42
6 months		
PSTD depth (mean ± SD) (mm)	0.29 ± 0.47*	0.89 ± 0.90
Complete PSTD coverage (%)	64.3	42.9
PD (mean ± SD) (mm)	2.04 ± 0.41	2.04 ± 0.31
CAL (mean ± SD) (mm)	2.32 ± 0.54*	2.93 ± 0.92
KMW (mean ± SD) (mm)	3.61 ± 1.06*	2.61 ± 1.36
AM (mean ± SD) (mm)	1.57 ± 1.27*	0.64 ± 1.12
MT (mean ± SD) (mm)	2.62 ± 0.52	2.41 ± 0.35
Baseline – 6 months		
Mean PSTD coverage (mean ± SD) (%)	87.85 ± 20.58*	64.40 ± 36.14
PSTD depth reduction (mean ± SD)	2.18 ± 0.95*	1.46 ± 0.80
CAL gain (mean ± SD) (mm)	2.29 ± 0.96*	1.46 ± 0.84
KMW gain (mean ± SD) (mm)	1.64 ± 0.84*	0.82 ± 1.10
AM gain (mean ± SD) (mm)	1.14 ± 0.84*	0.36 ± 1.05
MT gain (mean ± SD) (mm)	1.44 ± 0.46*	0.99 ± 0.52

Legend. AM: attached mucosa. CAL: clinical attachment level. KMW: keratinized mucosa width. MT: mucosal thickness. PD: pocket depth. PSTD: peri-implant soft tissue dehiscence. SD: standard deviation. *denotes a p-value < 0.05

4.2.3 Volumetric outcomes

Volumetric outcomes are depicted in the Table 3. The ROI between the two groups was not statistically significant different at any comparison ($p>0.05$). After 3 months, sites allocated to CAF showed a statistically significant higher Vol and ΔD than sites allocated to TUN. At the last recall, the CAF and TUN showed a mean Vol of 75.90 mm^3 and 37.25 mm^3 ($p<0.01$), and a mean ΔD of 1.01 mm and 0.53 mm ($p<0.01$), respectively (Table 3).

Table 3. Volumetric changes at 3 and 6 months obtained from the superimposition of the STL files at the different follow-up time points to the digital models at baseline.

Outcome measure	CAF + CTG (N=14)	TUN + CTG (N=14)
Baseline – 3 months		
Vol (mean ± SD) (mm^3)	63.42 ± 24.13*	41.03 ± 20.86
ΔD (mean ± SD) (mm)	0.91 ± 0.25 *	0.61 ± 0.25
Baseline – 6 months		
Vol (mean ± SD) (mm^3)	75.90 ± 37.29 *	37.25 ± 18.47
ΔD (mean ± SD) (mm)	1.01 ± 0.41 *	0.53 ± 0.22

Legend. SD: standard deviation. Vol: volume change in mm^3. ΔD: mean thickness of the reconstructed volume in mm. *denotes a p-value ≤ 0.01

4.2.4 Ultrasonographic outcomes

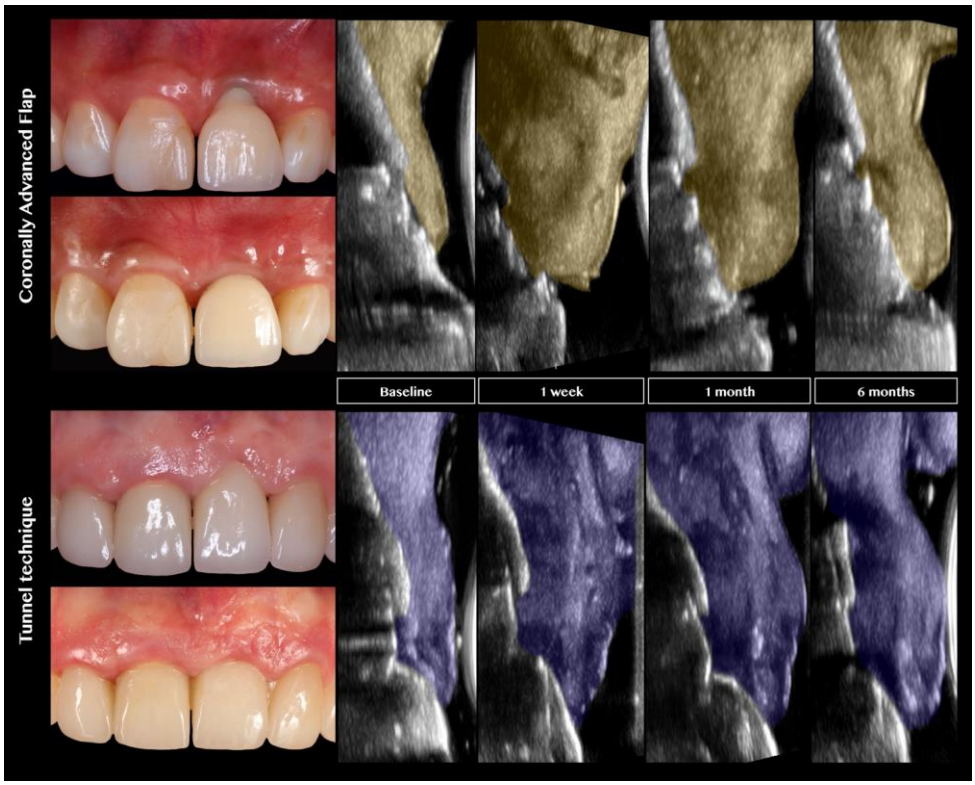
No difference between the two groups was present at baseline for the ultrasonographic outcomes of interest. At the sites allocated to CAF, the distance between the crown margin and the bone crest measured with ultrasonography was 6.34 ± 2.42 mm, while with the intrasurgical correspondent measurement was 6.75 ± 2.37 mm (Supplementary Figure 3 of the Appendix).
A substantial increase in UMT between baseline and the other time points was observed in both groups (Figure 6 and Table 4). A superior mean UMT gain at 3 mm was found for the CAF over the TUN group (1.59 mm vs 1.10 mm, $p=0.01$) at the last follow-up, while no differences were noted for UMT gain at 1 mm, nor at 5 mm ($p>0.05$). The mean CM-CB change from baseline to 6 months was -0.72 mm and -0.21 mm for CAF and TUN, respectively, indicating a higher buccal bone resorption following CAF than TUN ($p<0.01$). A similar result was also observed when evaluating BBD changes at 6 months (-0.67 mm and -0.18 mm for CAF and TUN, [$p<0.01$]). Both groups exhibited an increase in STH after 6 months, with CAF-treated implants showing a greater mean STH change than sites allocated to TUN (2.44 mm vs 1.43 mm, $p<0.01$) (Table 4).

Table 4. Ultrasonographic outcomes at baseline and 6 months.

Outcome measure	CAF + CTG (N=14)	TUN + CTG (N=14)
Baseline		
UMT 1 (mean ± SD) (mm)	0.92 ± 0.33	1.12 ± 0.31
UMT 3 (mean ± SD) (mm)	1.28 ± 0.29	1.56 ± 0.47
UMT 5 (mean ± SD) (mm)	1.53 ± 0.50	1.66 ± 0.90
BBD (mean ± SD) (mm)	3.51 ± 2.38	2.57 ± 2.05
CM-CB (mean ± SD) (mm)	6.34 ± 2.42	5.30 ± 2.28
STH (mean ± SD) (mm)	4.40 ± 2.36	3.87 ± 2.46
6 months		
UMT 1 (mean ± SD) (mm)	2.12 ± 0.58	2.08 ± 0.63
UMT 3 (mean ± SD) (mm)	2.86 ± 0.59	2.66 ± 0.61
UMT 5 (mean ± SD) (mm)	3.06 ± 0.75	3.04 ± 0.95
BBD (mean ± SD) (mm)	4.18 ± 2.37	2.75 ± 2.01
CM-CB (mean ± SD) (mm)	7.06 ± 2.53	5.51 ± 2.31
STH (mean ± SD) (mm)	6.84 ± 2.66	5.29 ± 2.27
Baseline – 6 months		
UMT 1 gain (mean ± SD) (mm)	1.21 ± 0.47	0.96 ± 0.63
UMT 3 gain (mean ± SD) (mm)	1.59 ± 0.49*	1.10 ± 0.45
UMT 5 gain (mean ± SD) (mm)	1.53 ± 0.70	1.38 ± 1.06
BBD change (mean ± SD) (mm)	-0.67 ± 0.40*	-0.18 ± 0.20
CM-CB change (mean ± SD)	-0.72 ± 0.36*	-0.21 ± 0.24
STH change (mean ± SD) (mm)	2.44 ± 0.93*	1.43 ± 0.71

Legend. BBD: ultrasonographic buccal bone dehiscence. CM-CB: distance between the crown margin and the crestal bone. STH: supracrestal tissue height. UMT 1: ultrasonographic mucosal thickness measured 1 mm apical to the soft tissue margin of the implant. UMT 3: ultrasonographic mucosal thickness measured 3 mm apical to the soft tissue margin of the implant. UMT 5: ultrasonographic mucosal thickness measured 5 mm apical to the soft tissue margin of the implant. *denotes a p-value < 0.01

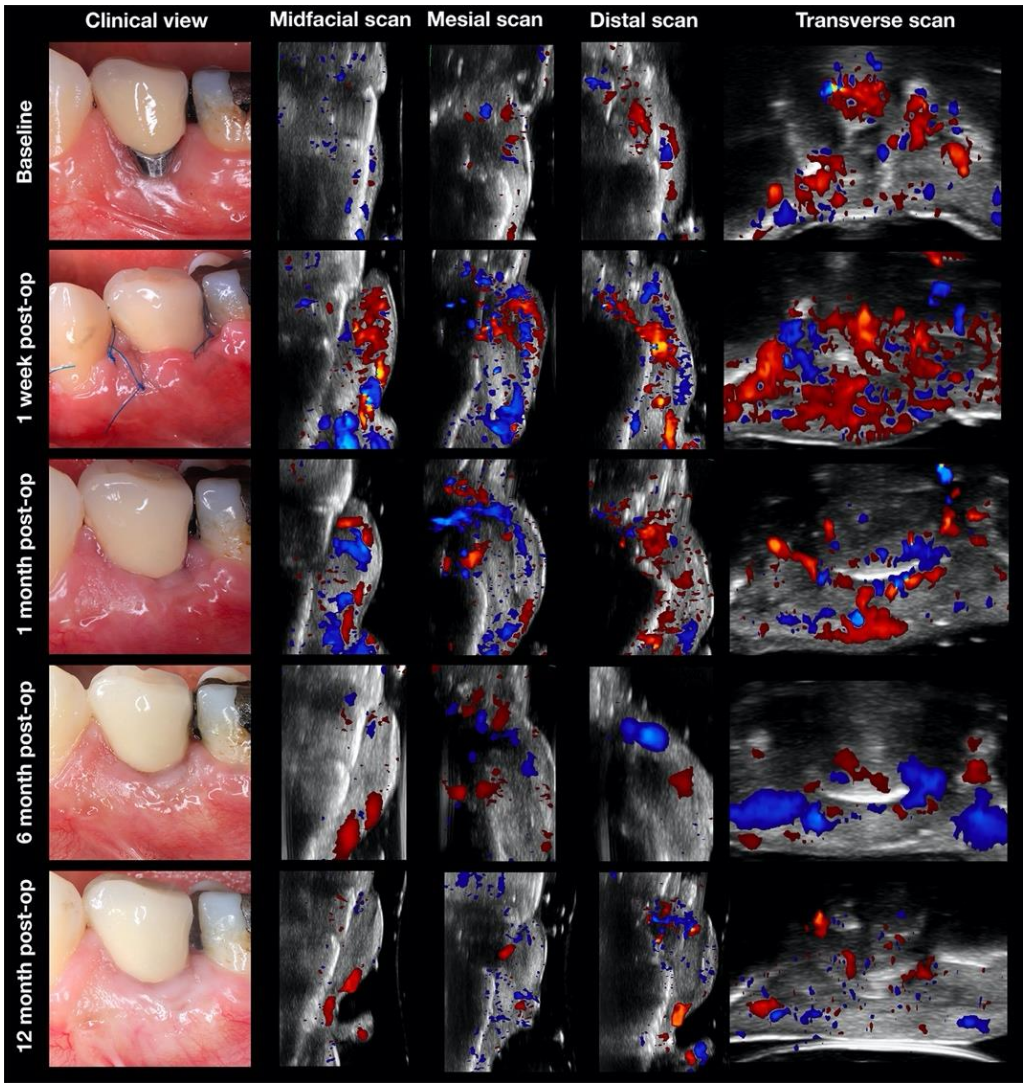
Figure 6. Ultrasonographic soft tissue changes over time. The orange areas highlight the soft tissue component on midfacial ultrasonographic scans obtained at baseline, 1 week, 1 month and 6 months in an implant site treated with coronally advanced flap and connective tissue graft. The blue areas in the ultrasonographic scans display the soft tissue changes in the midfacial aspect of an implant allocated to tunnel technique with connective tissue graft.



4.2.5 Ultrasonographic Power Doppler Outcomes

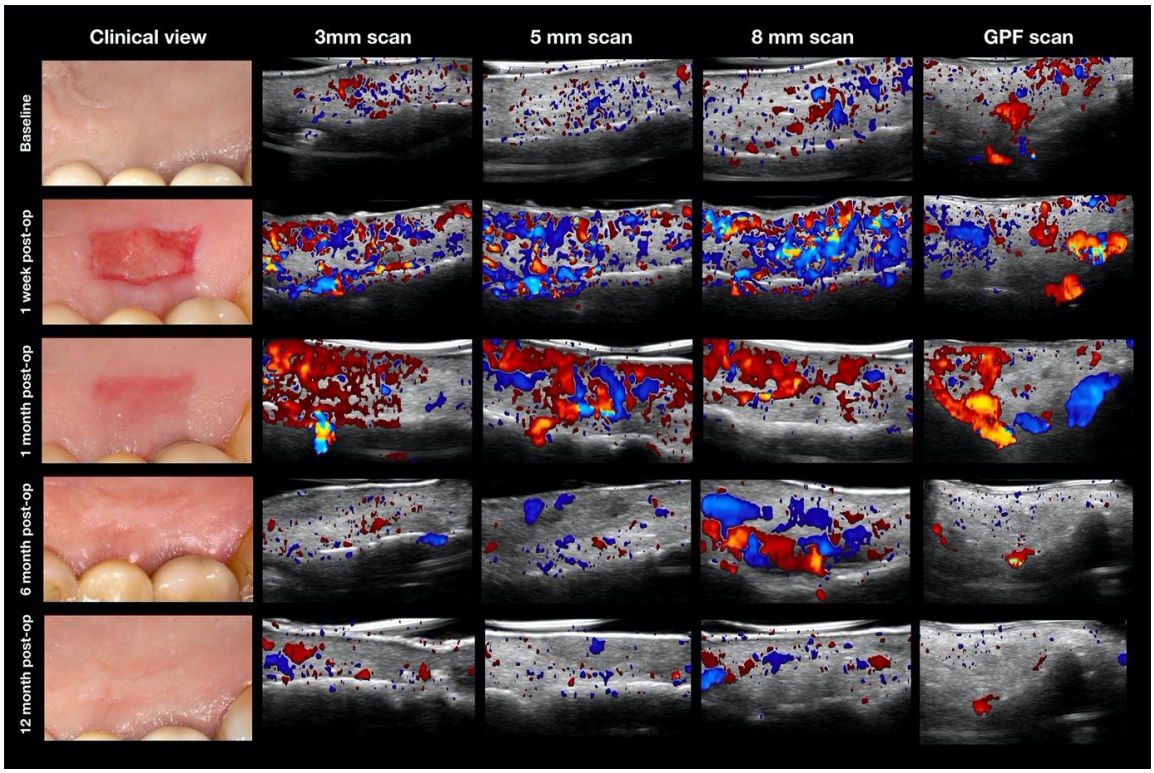
Tissue perfusion changes at implant sites. In the midfacial scan, an increase in CV_m of 199% was observed compared to baseline at the 1-week follow-up. The CV_m increase in the mesial, distal and transverse scans were 102%, 95.6% and 163%, respectively, compared to baseline. The CV_m increase at 1 month was similar to the one observed at 1 week in all the scans. At the 6- and 12-month follow-up, CV_m was found to be lower than baseline (Figure 7). A similar trend was observed for CP_m change over time (Supplementary Table 7 of the Appendix).

Figure 7. Ultrasound color mode at the implant site at the midfacial, transverse, mesial and distal scan, showing the variation in color velocity at baseline, 1-week, 1-month, 6-month and 12-month follow-up [113].



Tissue perfusion changes at palatal donor sites. At the 1-week follow-up, the CV change was 146% at the 3-mm scan, while the 5-mm and 8-mm scan showed a CV increase of 179% and 222%, respectively compared to baseline. The CV increase at 1-month was found to be still higher than baseline values in all the scans. At the 6- and 12-month recalls, similar CV change were found in the 3-, 5- and 8-mm scans, with minimal differences compared to baseline CV. The CV at the GPF area showed an increase of 50.1% after 1 week, 40.8% after 1 month, 11.8% after 6 months and 4.81% after 12 months (Figure 8). CV and CP over the 12-month observation period at the palatal site are reported in the Supplementary Table 8 of the Appendix.

Figure 8. Ultrasound color mode at the palatal sites at different time points. An increase in blood volume was observed in all the scans (3 mm, 5 mm, 8 mm and greater palatal foramen [GPF]) at the 1-week and 1-month follow-up, compared to baseline [113].



4.2.6 Esthetic outcomes

The professional esthetic evaluation using the IDES showed a mean final score of 7.00 vs 4.93 points for the CAF and TUN groups, respectively (p=0.03). Regarding the individual component of the IDES, CAF-treated sites obtained a statistically significant superior mean score for the level of the soft tissue margin (3.71 vs 2.14 points, p=0.03), while a significantly higher peri-implant mucosa appearance was observed in sites treated with TUN compared to CAF (0.79 vs 0.36 points, p=0.02) (Supplementary Table 9 of the Appendix).

5. DISCUSSION

5.1 Prevalence and risk indicators for peri-implant soft tissue dehiscences

The present cross-sectional study, with the aid of clinical and ultrasonographic measurements, identified the prevalence of dental implants with PSTD, as well as risk indicators for the presence of this condition. Based on the definition of PSTD proposed by Burkhard et al. [84], and later on adopted by Zucchelli and coworkers [43, 72], using the contralateral homologous tooth as a reference, it is not unexpected that most of the implants evaluated in our study displayed PSTD (56.8%). On a patient-level, it was found that having at least one implant with PSTD was more common than having implants without this condition (54.2% vs 45.8%). It should be highlighted that our population cohort included patients which had implants placed both in a private practice and in a university setting, which would increase the generalizability of our findings.

Previous studies defined soft tissue dehiscence as the exposure of the prosthetic abutment or the implant neck [44, 62, 63], and therefore a comparison between our findings and these studies was not attempted. Given the fact that PSTD is an esthetic complication often associated with esthetic concerns/complaints from patients [44, 52], it is reasonable to assume that the definition of PSTD should not solely include cases with exposure of the abutment/implant fixture but should also include conditions characterized by an implant-supported crown longer than the clinical crown of the homologous contralateral tooth. In this view, the present study represents the first report investigating the prevalence of PSTDs, together with their types, classes, and subclasses, according to the recent classification by Zucchelli et al. [43].

We observed that most of the PSTDs are characterized by a crown longer than the homologous contralateral tooth (84%), with or without concomitant exposure of the abutment/implant fixture (58% and 26% of all the PSTD cases, respectively). This finding has implications on treatment of these defects, since the correction of PSTDs with inadequate crown length requires crown removal in combination with the prosthetic-surgical technique or the submerge approach [43, 44, 114]. Clinicians are therefore advised that crown removal is necessary in most of the PSTD treatments. We also found that the exposure of the abutment/implant fixture was present in 74% of sites with PSTDs. Aside from patient esthetic concern, the exposure of the implant surface, especially if rough, may facilitate plaque accumulation on the implant fixture which is considered the main risk factor for peri-implantitis [92, 115]. While the main indication for the treatment of PSTDs without abutment/implant exposed remains patient esthetic concern [24, 43, 116], PSTDs with rough implant surface exposed to the oral cavity should be treated for maintaining peri-implant health and preventing future complications [107, 116, 117]. It is important to further highlight that having a crown with an inadequate length and abutment/implant fixture exposed are common findings, with an overall prevalence (considering all the implants examined in our study) of 47.7% (PSTD with inadequate crown length) and 42% (PSTD with exposure of the abutment and or implant fixture).

The multivariate analysis demonstrated that having an adjacent implant, the time in function of the implants, KMW, MT and BBD are risk indicators for PSTD. Previous studies concluded that limited KMW was associated with PSTD [10, 62, 63] and our findings further confirm this correlation. However, readers should bear in mind that as this study was conducted

in a cross-sectional design, it was not conducted and does not allow for a direct exploration of causality, thus whether a narrow band of KMW is a risk factor for PSTD or a consequence of this condition has yet to be elucidated with prospectively and longitudinal studies. It is reasonable to assume that there are scenarios in which inadequate KMW can contribute to the development of this condition, and other cases in which KMW becomes narrow as a result of the PSTD.

The use of ultrasonography allowed us to evaluate BBD and BBT which otherwise could only be assessed with cone-beam computed tomography (CBCT), which involves a dose of radiations that may not be recommended for an observational study. Ultrasonography may also be considered the technology of choice for assessing MT, given the limitations of transgingival horizontal probing (needing anesthesia, having patient discomfort and reduced accuracy), optical scanners (needing at least two time points, unless the STL file were combined with the DICOM scan from the CBCT [118]), and CBCT alone (radiation, and inaccuracy) [108, 119]. Nevertheless, it has to be mentioned that a method's error of 0.015 mm and 0.08-0.2 mm was observed for MT and BBD, respectively, when obtained with US compared to direct measurements [98]. Interestingly, US was found to be more accurate than CBCT in identifying crestal bone level and MT [98].

We observed that BBD has an OR for PSTD of 1.41. In other words, each millimeter increase in the distance between the crestal bone and the implant platform, raises the odds of having a PSTD by a factor of approximately 41%.

Previous studies investigated the effect of BBD and BBT on the position of the mucosal margin [2, 120, 121]. Nevertheless, there is no consensus in the recent literature [5]. A recent animal study reported that dental implants with BBT < 1.5 mm were more often associated with PSTD compared to implants with thick buccal bone [120]. However, other authors did not find a correlation between BBT and PSTD, even for implants missing the buccal bone wall [62, 122]. In our analysis, when other factors were taken into account, BBT was not found to be associated with PSTD. It may be reasonable to assume that buccal bone resorption in the vertical (BBD) - but not horizontal (BBT) – aspect can negatively affect the stability of the mucosal margin.

We also observed an inverse correlation between MT and PSTD, corroborating the notion that a thicker mucosa can improve the stability of the peri-implant mucosal margin and the esthetic outcomes [24, 123]. This concept has previously been proven in the natural dentition [124] and seems to be valid also at implant sites. In addition, a recent network meta-analysis from our group further highlighted the importance of the dimension of the peri-implant soft tissues, demonstrating that MT augmentation has also beneficial effects on marginal bone level stability [117].

5.2 Treatment of Peri-implant soft tissue dehiscences

Limited evidence – mainly from case reports and case series – is available on the efficacy of different treatments on peri-implant soft tissue dehiscences [24, 125]. To the best of our knowledge, this is the first randomized clinical trial reporting the outcomes of PSTD treatment with two different approaches in combination with autogenous connective tissue graft. The rationale for this comparison is that coronally advanced flap and tunnel technique are considered the two most effective and performed root coverage techniques in natural dentition [126].

Previous studies reported a mean PSTD coverage ranging from 28 to 89.6% when CAF was performed without removing the implant-supported crown [52, 83, 84]. Other authors reported mean PSTD coverage from 88% up to 96.3% when removing the implant-supported crown [72, 127]. Nevertheless, a comparison with our results may not be appropriate given the heterogeneity of PSTDs included and the different study design. In order to facilitate generalizability of our outcomes and comparison with future trials, we utilized the recent classification of PSTDs [43]. Also, to reduce possible confounding variables between CAF and TUN, our protocol did not allow for crown removal. This aspect may have limited the amount of mean and complete PSTD coverage, but provides a valuable information for clinicians and patients by reporting the outcomes of the treatment of PSTDs treatment when the implant-supported crown is not removed and replaced. The decision of removing the crown prior to the surgical treatment of PSTDs should be based not only the characteristics of the crown itself and on the soft tissue dehiscence, but also patient's demands and expectations [24, 44, 125].

Our results showed that CAF + CTG obtained a statistically significant higher mean PSTD coverage and complete PSTD coverage than TUN + CTG after 6 months (87.9 vs 64.4% and 64.3 vs 42.9%, respectively). CAF was also associated with significantly greater KMW gain, AM gain and MT gain compared to TUN. While these two techniques have been shown to provide overall similar root coverage outcomes in natural dentition [126, 128], the reason for the different results in our study is open to speculation. First, it should be considered that peri-implant mucosa resembles more a scar tissue than the health periodontium [77, 129]. The detachment of the flap from the implant surface and the surrounding bone/periosteum may pose some challenges with TUN, especially when crown removal is not an option. Similarly, proper flap release and the elimination of muscle fibers and residual tension is probably more difficult in presence of bulky crowns that do not allow tunneling blades to entering the sulcus with the desired angulation. These challenges do not apply for CAF. Another factor that may have contributed to the higher outcomes observed for CAF, is the possibility of elevating the flap, preparing some areas split-thickness and other full-thickness. It has been advocated that the midfacial area of the PSTD should be raised split thickness in order to leave some connective tissue fibers over the exposed (and not contaminated) implant surface to facilitate the attachment of the CTG [125].

The CAF approach also allows for the stabilization of the graft to the de-epithelialized anatomical papillae and also to the adjacent or apical periosteum. This may have provided a greater stability of the CTG during the healing in the sites allocated to the CAF compared to sites treated with TUN, where the graft was stabilized to the flap only.

Interestingly, the regression analysis found that the mean PSTD coverage is associated not only with the treatment approach, but also with the subclass. PSTDs subclass b, characterized by at least one papilla less than 3 mm in height (but not flat), negatively affected the amount of PSTD coverage. Although this finding is in line with the classification system of gingival recessions based on the interproximal clinical attachment level [130], this is the first time that the recent PSTD classification [43] has been shown to also have a prognostic value.

The present study adopted three different methods for assessing mucosal thickness/ profilometric changes. The superiority of CAF compared to TUN in terms of volume/thickness gain was demonstrated through transmucosal piercing, intraoral optical scanning and ultrasonography. Although the importance of MT on peri-implant health and esthetics have

been emphasized [4, 41, 117], there are not uniform guidelines for assessing volumetric changes at implant sites [108]. One of the most utilized methods involve the piercing of the soft tissue with needles or endodontic files. However, this approach has several limitations, including patient discomfort and the need for customized stents, as well as a questionable accuracy in measuring tissue thickness due to possible bending of the needle [108]. Optical scanning is a valuable and non-invasive approach for performing volumetric comparisons between different time points [107, 131, 132]. However, this method can only provide volumetric changes and not the actual measurement at a specific time point. In addition, optical scanners capture the contour of the mucosa only, and the volumetric outcomes from the STL superimposition cannot differentiate between changes in the soft and/or hard tissue. Ultrasonography is a non-invasive and reliable technology for assessing peri-implant structures [93, 99, 113]. In our study, ultrasonography was also utilized for quantifying not only soft tissue thickness (MT and STH), but also buccal bone changes following the interventions. The reliability of ultrasonography in assessing buccal bone position has been previously demonstrated [98, 133, 134], with our intraoperative findings further corroborating this conclusion. Interestingly, PSTDs treated with CAF exhibited an average buccal bone loss of 0.7 mm after 6 months, which was significantly higher compared to TUN-treated sites (0.2 mm on average). While classic studies already highlighted that a certain amount of bone resorption should be expected after raising split or full-thickness flaps in natural dentition [135, 136], the fate of buccal bone following CAF and TUN at implant sites have never been investigated in a clinical trial so far. It can be speculated that the negligible bone loss observed for TUN is due to its more conservative approach that preserves the integrity of the papillae and the vascularization of the flap [102, 103, 126]. On the other hand, the greater access provided by the CAF and the incisions at the level of the interproximal soft tissue may have caused more damages to the peri-implant vasculature, resulting in a more extensive and prolonged inflammatory phase during the healing, with consequent higher bone resorption than TUN-treated sites. Future studies are needed to further explore this aspect.

Lastly, it has to be mentioned that both treatment approaches resulted in an increase in STH, with CAF showing a significantly higher STH gain than TUN (2.44 mm vs 1.43 mm, respectively). The effect of STH on peri-implant health has been largely debated without reaching a definitive conclusion [5, 137]. While it has been shown that a reduced STH is associated with higher marginal bone loss after implant placement [5, 138, 139], a recent case-control study demonstrated that implants with excessive STH (≥ 3 mm) had delayed and incomplete resolution of peri-implant mucositis as compared to implants with shallow STH [140]. Moreover, in subjects with history of periodontal disease, the risk for peri-implantitis was found to increase 1.5 times for each mm of STH increase[141]. Nevertheless, readers should bear in mind that other implant and restorative factors could play a role on the manifestation and resolution of peri-implant diseases [137, 142].

6. NEW FINDINGS

1. PSTDs are commonly observed in the esthetic region. Factors associated with this esthetic complication include presence of an adjacent implant, increased time in function of the implant, higher buccal bone dehiscence, lower KMW and MT.
2. CAF was found to be more effective than TUN for the treatment of class II PSTDs, when combined with CTG, in terms of mean and complete PSTD coverage.
3. CAF resulted in a higher CAL gain, KMW gain, AM gain and MT gain than TUN at 6 months.
4. Superior 3D volumetric gain and ultrasonographic MT gain were observed for CAF over TUN.
5. Ultrasonography showed to be a valuable tool for characterizing the peri-implant phenotype and assessing PSTD treatment outcomes, including tissue perfusion changes over time not only around implants but also at the palatal donor sites.

7. SUMMARY

Esthetic complications of dental implants in the esthetic zone can have a major negative impact on patients' quality of life and perception of implant therapy. Our group conducted a cross-sectional study aimed at assessing the prevalence of PSTDs in the esthetic region, as well as risk indicators associated with this condition, utilizing clinical and ultrasonographic measurements. It was observed that the prevalence of PSTD was 54.2% and 56.8% on a patient and implant level, respectively. The most frequent type of PSTD was the one characterized by having both an implant-supported crown longer than the clinical crown of the homologous tooth and a visible abutment/implant fixture exposed to the oral cavity. The multi-variate analysis showed that the presence of an adjacent implant, a longer time of the implant in function, limited MT, reduced KMW and increased BBD were significantly associated with the presence of a PSTD.

Next, we conducted a randomized controlled trial aimed at evaluating two different approaches, for the treatment of class II PSTDs. Twenty-eight subjects presenting PSTDs were enrolled and randomized to receive CTG either with CAF or TUN. The percentage of mean PSTD coverage at 6 months was set as primary outcome. Secondary endpoints included the frequency of complete PSTD coverage, changes in peri-implant soft tissue phenotype, profession esthetic evaluation and ultrasonographic tissue perfusion changes over time.

At 6 months, the mean PSTD coverage of CAF was statistically significantly higher than the one observed at sites treated with TUN. Linear regression analysis demonstrated that the treatment and the type of PSTD subclass were significantly correlated to the mean PSTD coverage. In other words, subclass "a" PSTDs and sites allocated to CAF + CTG obtained higher outcomes than subclass "b" PSTDs and sites treated with TUN + CTG.

CAF-treated sites also obtained a significantly higher frequency of complete PSTD coverage, together with significantly greater keratinized mucosa width gain, increase in mucosal thickness gain and volumetric gain. Ultrasonographic analysis revealed a lower buccal bone resorption for TUN over CAF.

Ultrasonographic power doppler showed tissue perfusion variation at the grafted implant sites and palatal donor sites. Blood flow increased in both surgical sites at the earliest time points until reaching baseline levels or even lower tissue perfusion values at the last follow-up visit.

These two studies highlight the important of peri-implant phenotype, showing the risk indicators for PSTDs that should be addressed during implant placement for reducing the chance of developing implant esthetic complication that, however, can be effectively treated with a bilaminar approach involving CTG, preferably with CAF.

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