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Peri-implant soft tissue augmentation with palate subepitelial connective tissue graft compared to porcine collagen matrix: A randomized controlled clinical study and histomorphometric analysis

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Abstract
The objective of this study was to compare the connective tissue graft with Mucograft® collagen matrix, used to increase the peri-implant soft tissue, regarding the keratinized mucosa and number of fibroblasts between the grafted areas and the areas not inherent to the grafts. 12 patients were treated with 2 implants, one with connective tissue and the other with collagen matrix. Measurements were obtained before grafts and at uncoverage (2-3 months), biopsies were performed for fibroblast counting. Was an increase in the width and thickness of the KM for both grafts tested, the width of the KM was significantly higher in the connective tissue (P<0.014) the thickness was similar for both. There was no statistically significant difference in the number of fibroblasts between the two types of grafts. Conclusion: Both grafts resulted in increased the width and thickness of KM while maintaining similar fibroblast counts for both areas. The connective tissue exhibited a statistically significant superiority in increasing the width of the KM, being the better indication for areas with KM < 2mm.

Keywords: Collagen, connective tissue, dental implants

1. Introduction

Soft tissue graft procedures are a usual and predictable solution for clinical situations where their absence compromises the health or aesthetics around of teeth or dental implants [1, 2]. The autogenous graft presents in the current literature the best clinical and histological results in soft tissue regeneration techniques and is considered the gold standard to be followed, however, it requires tissue removal from a donor area. Techniques and materials are being tested to avoid this additional surgical procedure [3, 4]. CM have recently been introduced for MK augmentation and have shown promising results in preclinical and clinical studies, but more information is needed to substantiate their clinical efficacy [3-5]. Geistlich Mucograft® is a three-dimensional porcine CM and has been developed for the regeneration of soft tissues as an alternative to autogenous soft tissue grafts, especially in cases of root coverage. A significant gain in the KM band was found when performing root coverage using total thickness Coronally Advanced Flap (CAF) technique plus Geistlich Mucograft®. [6] Geistlich Mucograft® xenogeneic CM has a bilaminate structure, one a compact and resistant with slower resorption and other one a spongy structure that stabilizes the blood clot and allows the penetration of soft tissue cells. The proliferation and viability of human gingival fibroblasts cultured on Geistlich Mucograft® was demonstrated in an in vitro study, in this experiment the CM Mucograft® presented cellular compatibility, being an option for a scaffold whenever it is required [7]. In dog extraction sockets, preliminary results suggest that the association Mucograft® plus Bio-Oss Collagen®, an bovine xenograft, may be a valuable method to be used for ridge preservation. The combination Mucograft® plus Bio-Oss Collagen® was associated with an increased osseous deposition in the alveolus post-extraction in comparation its non-use [8]. In an animal model in pigs to assess the soft tissue response to a CM, showed no inflammatory adverse reaction and optimal integration within 30 days postoperative [5]. A new design of CM, Mucograft® Seal, foi development for use in combination plus Bio-Oss Collagen® to be used for ridge preservation in the post-extraction. With the aim to compare a

porcine CM with a free gingival punch graft with respect to size, invagination, and color of resulting soft tissue scar, the authors suggest that use bovine bone mineral and CM leads to less scar tissue formation when compared with bovine bone mineral and free gingival punch grafts from the palate [9].

The objective of the present study was to compare the clinical results of the autogenous CTG with the Mucograft® xenogeneic CM, used to increase the peri-implant soft tissue, regarding the width and thickness of the KM and histomorphometric findings relating the number of fibroblasts between the GA and the ANIG.

2. Materials and Methods
This was a randomized controlled clinical and histomorphometric study.

2.1 Participants
The study was approved by the research ethics committee of the São Leopoldo Mandic School under the number: 2.485.441.

Fifteen adult patients of both sexes, older than 20 years, who had no systemic restrictions to the surgical procedure, non-smokers with at least 2 missing teeth in the posterior mandible were selected in the period between February 1, 2018 and June 1, 2018. Patients were assessed for periodontal health, and when necessary previously treated. Prosthetic planning was performed and bone availability was assessed using computed tomography. It was selected for the study only cases where there was no need for bone reconstruction. We adopted as exclusion criteria any general contraindications for implant surgery; pregnant or lactating; untreated periodontitis; bruxism or clenching severe; immunosuppressed, history of irradiation of the head and neck area; uncontrolled diabetes; heavy smoker (> 10 cigarettes / day); poor oral hygiene and low motivation; use of bisphosphonates; substance abuse like alcohol and other drugs and psychiatric disorders. During the initial phase there were 3 not following cases, with 12 patients remaining until the end of the study (Table 1).

Table 1: Sample description

Patients number	12
Mean age (interval)	46 (28-68)
Gender (male/female)	3/9
Mean time for implants uncoverage - month (interval)	3 (2-3)
Number of implants	24
Number of connective tissue grafts	12
Number of Mucograft® collagen matrix	12

2.2 Clinical measurements
To measure the width of the keratinized tissue band, a dry-point compass and a digital caliper were used, marking the distance between the lingual and buccal mucogingival lines at the center of the mesio-distal prosthetic space of the future prosthetic crown. To measure supracrestal soft tissue thickness, after anesthesia, a puncture with a short carpule needle with an endodontic rubber cursor was performed in the center of the future prosthetic crown measured with surgical guides, marking the depth of the tissue while the needle touched the bone crest (Figure 1). The clinical measurements were performed in 2 moments, during the placement of the implants (Step 1) and during the implants uncoverage (Step 2), when biopsy material was collected as well placed healing abutments on the implant.

Fig 1: Width and thickness measurement

2.3 - Surgical protocol
Two implants were placed in each patient, one with CTG of the palate and the other with a Geistlich Mucograft® Seal CM for supracrestal covering in the implant position, the graft materials were fixed in a sub-periosteal level through the elevation of a full-thickness flap. The Mucograft® Seal was designed for sealing orifice of the extraction socket in alveolar ridge preservation, this format was chosen in this study to allow a better standardization of diameter and thickness with the CTG autogenous. The implants placed were Titamax Cortical Cone Morse (Neodent, Curitiba, Brazil), compatible with available bone volume and sufficient to meet prosthetic needs. The implants were performed following the surgical two-time protocol, in the first step (S1) it was performed the primary closure of the wound during the implant placement and tissue reconstruction. For removal of the CTG from the palate, the donor area was limited to the posterior canine region to the distal region of the first molar, maintaining a 2 mm safety of the palatine gingival margin of the adjacent teeth. For standardization of graft diameter and thickness, we used a circular scalpel (punch) 8 mm in diameter with marking 4 mm of depth, the removal all of the epithelial tissue was performed using a scalpel blade number 15, promoting dimen sions very similar to the CM (Figure 2).
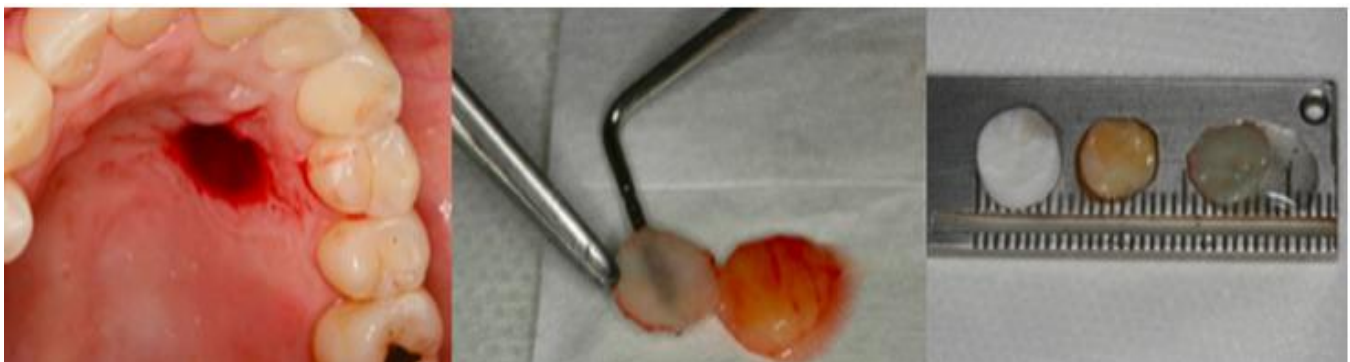

Fig 2: Removal of the CTG from the palate, similar to the CM.

Fig 3: Sutures of fixation of the grafts on the recipient area.

To fix the graft two simple sutures were made with mononylon 5.0, one to vestibular flap and the other to lingual flap, material was grafted onto the alveolar bone crest at the region of the implants and the flaps where closed primarily with simple sutures completely covering the grafts (Figure 3). The removal of the sutures was performed between 7 and 10 days after surgery.

2.4 - Sample collection
In the second step (S2), after 2 to 3 months, it was performed implants exposure and collection of biopsy material and placed healing abutments. At the time of reopening of the implants (S2), new measurements of the width and thickness of the MK were made. Linear incisional biopsies with a standardized thickness of 1.5 mm were performed double blade scalpel, in the grafted place, and then immersed in 10% formalin solution for histological analysis (Figure 4).

Fig 4: Linear incisional biopsies with a standardized thickness of 1.5 mm and then immersed in 10% formalin solution.

Healing abutments with height compatible with the tissue thickness were installed in the 2 implants with posterior closure of the surgical wound with simple sutures, which were removed between 7 and 10 days after surgery. 2 to 3 months after exposure of the implants, the prosthetic abutments were fixed and the cases were finished with screw driven ceramic metal crowns (Figure 5).

Fig 5: The prosthetic abutments fixed, cases finished with ceramic metal crowns.

2.5 Sample processing
The samples were processed through alcohol, xylene and paraffin baths. After dehydration and diaphanization the tissues were included in paraffin blocks, and then serial cuts of 4 μm thickness were performed on all samples in the microtome. The slides were dewaxed, hydrated and stained by the hematoxylin-eosin method. The images of the microscope slides were obtained with a Leica DM 500 optical microscope using the Leica Application Suite software (LAS EZ) (Figure 6). The counting of the fibroblasts on the slides was performed with the aid of an Image-Pro® image analyzer program (Media Cybernetics, Silver Spring, MD, USA). In all slides, the counting was performed in two distinct areas, an area close to the epithelium with no grafted material present, ANIG, and an area close to the periosteum, within the graft area, GA, since the graft materials were fixed through the elevation of a full-thickness flap. (Figure 6).
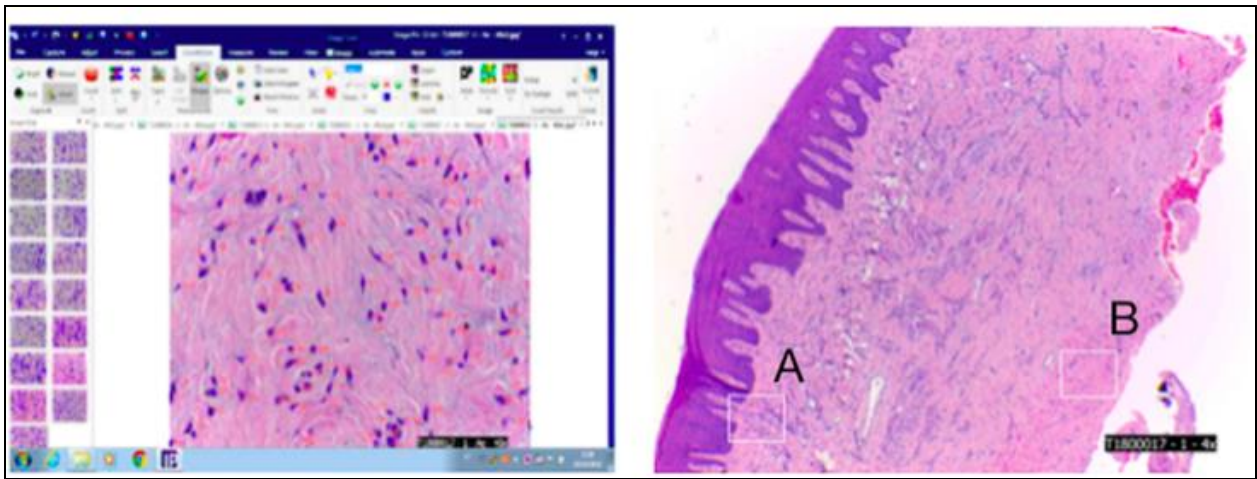
Fig 6: Image analyzer program and areas utilized, A - ANIG and B - GA.

2.6 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software, version 22.0. The normality of the variables was analyzed by the Kolmogorov-Smirnov test. Student's t-test was used to compare mean and standard deviation (SD) of variables with normal distribution. The independent samples t-test was used to compare the mean and SD of the variables width, thickness and number of fibroblasts per graft type. The comparison of the width and thickness variables in each time protocol (S1 and S2) was performed through the t-test of paired samples. The same test was used to compare the mean and SD of the number of fibroblasts per area (GA and ANIG). The level of significance was 5%.

3. Results

In this clinical and histomorphometric study in human, the histological analyzes showed complete complete CM resorption and replace by healthy connective tissue, revealed also healthy strands of collagen and nothing portion of the CM could be detected in anything samples. Blood vessels were present and no inflammatory infiltrate could be seen in Step 2 (2-3 months) in both grafted materials. The counting of the number of fibroblasts from the ANIG and the GA was performed at random, the histological sections showed similarities, in Step 2, for the collagen chains, distribution and number of fibroblasts in all the samples (Figure 7).
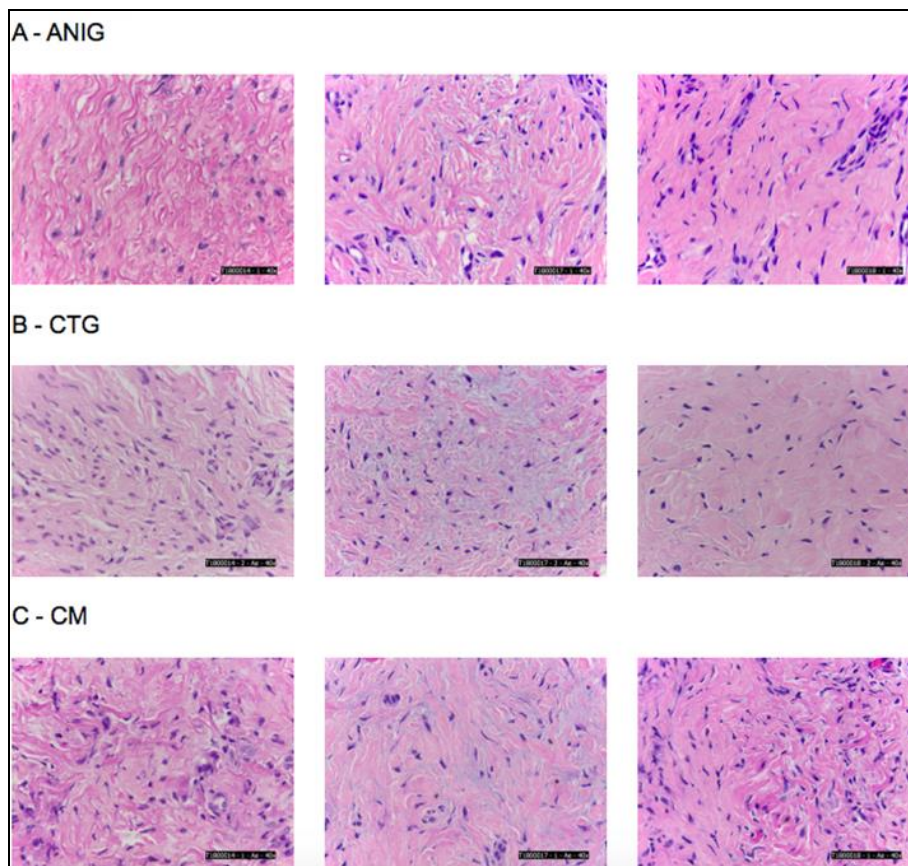

Fig 7: Histological analyzes for: A - ANIG, B - CTG and C – CM

Considering the variables of clinical measurements, width and thickness of the KM, we could observe a statistically significant increase of these measures from S1 to S2, in both CTG and CM (Table 2).

Table 2: Clinical measurements of CTG and CM on first and second steps

Clinical measurements	S1 Mean (SD)	S2 Mean (SD)	
CTG (n=12)			
Width	3,21 (0,71)	4,41 (0,55)	<0,001
Thickness	2,05 (0,33)	2,98 (0,50)	<0,001
CM (n=12)			
Width	3,00 (0,65)	3,67 (0,79)	0,003
Thickness	2,12 (0,33)	2,61 (0,43)	0,013

Si = first step, S2 = second step, SD = standard deviation, *P* = P-value, CTG = connective tissue graft, CM = collagen matrix.

When comparing the 2 types of grafts in S2, we observed that the width of the KM was higher in CTG, which presented 4.41 mm while the MC presented 3.67 mm, a statistically significant difference between the two types of grafts (*P*<0.014). Regarding keratinized mucosa's thickness, there was no statistically significant difference between the two grafts (*P*= 0.065), since the CTG showed 2.98 mm and the MGS, 2.6 mm (Table 3). Regarding the fibroblast counting in S2 we found a number of 108.00 (37.41) for CTG and 113.92 (43.96) for CM in the ANIG (*P*<0.726) while in the GA we found 120.58 (36.85) for CTG and 130.42 (43.04) for CM (*P*<0.554), with both countings showing no statistically significant differences in both ANIG and GA for both the types of grafts (Table 3).

Table 3: Clinical measurements of CTG and CM in first and second steps. Fibroblast count in ANIG and GA

Clinical measurements	CTG (n=12) Mean (SD)	CM (n=12) Mean (SD)	P
SI			
Width	3,21 (0,71)	3,00 (0,65)	0,444
Thickness	2,05 (0,33)	2,11 (0,34)	0,630
S2			
Width	4,41 (0,55)	3,67 (0,79)	0,014
Thickness	2,98 (0,50)	2,60 (0,43)	0,065
Fibroblasts			
ANIG	108,00 (37,41)	113,92 (43,96)	0,726
GA	120,58 (36,85)	130,42 (43,04)	0,554

S1 = first step, S2 = second step, *P* = P-value, SD = standard deviation, CTG = connective tissue graft, CM = collagen matrix, ANIG = areas not inherent to the grafts, GA = grafted areas

When comparing the number of fibroblasts between the ANIG and the GA, we did not observe a statistically significant difference between the two areas, both for CTG (*P*<0,365) and for MC (*P*<0.330) (Table 4).

Table 4: Number of fibroblasts **of** each graft - ANIG and GA

Graft type	ANIG Mean (SD)	GA Mean (SD)	P
CTG (n=12)	108,00 (37,41)	120,58 (36,85)	0,365
CM (n=12)	113,92 (43,96)	130,42 (43,04)	0,330
Total (n=24)	110,96 (40,03)	125,50 (30,51)	0,169

4. Discussion

To the best of our knowledge there is a limited number of clinical studies that have evaluated measurements change of the KM band, comparing the autogenous graft with biomaterials. There are also few studies which have evaluated the histological characteristic of the grafted area after the repair.

Long-term data on the impact of lack of KM on the health of peri-implant tissues are controversial. In a study evaluating two hundred and eleven patients with 967 dental implants, up to 15 years after implant placement, the results indicated that the presence of KM has a positive effect on peri-implant tissue health but did not appear to have any influence in maintaining the bone level [10]. On the other hand, in another recent study, fifty-four patients with 202 implants were evaluated for a period of 4 years regarding marginal bone level, plaque accumulation, tissue inflammation and brushing discomfort, was observed that marginal bone loss was higher in the group with narrow band of KM than in the group with broad KM; concluding that a width of KM ≥ 2 mm had a protective effect on the peri-implant tissues; and maintenance of the bone level [11].

In the present study, we observed an increase in the clinical measurements of the KM of the two grafts, both in width as in thickness, from implant placement and tissue reconstruction (Step 1) to the time of implants uncoverage (Step 2). Analyzing the outcome variables in Step 2, we found that the width enhancement was significantly greater in the CTG while the thickness increase was similar for both grafts. We found no statistically significant difference in the number of fibroblasts between the both grafts tested, nor between the among GA and ANIG.

As expected, due to the tissue grafting, there was an increase in the thickness of the KM band from S1 to S2. We also observed that there was an increase in the width of the KM, a finding also observed by Cardaropoli [12]. Who also compared the CTG to a xenogeneic CM in the treatment of gingival recession. Furthermore, Thoma [2]. in a systematic review, analyzed articles comparing autogenous grafts with biomaterials for soft tissue augmentation and reported an increase in clinical measurements during the periods evaluated. The authors suggested that more research should be done to investigate soft tissue regeneration techniques for reducing morbidity, increasing reliability and elimination of autogenous tissue.

In Step 2, we observed that the CTG presented a statistically larger increase in the width of the KM compared to the CM. Sanz [3] in a clinical study with free gingival grafts, with a 6-month control, comparing the apical displacement of the flap with connective tissue graft and the apical displacement of the CM flap, in areas with the same extension and keeping the grafts uncovered, found no statistically significant difference for the mean width of KM. He also found in both groups a marked tissue contraction, between 60% and 67%. In our study the grafts were interpositional, being covered and protected in the postoperative period, which may have contributed to a lower contraction during cicatrization, mainly in the connective tissue graft. Transplanted connective tissue has the ability to interfere in the differentiation of epithelial cells, and the characteristics of the gingival epithelium are determined by factors inherent to the underlying connective tissue [13]. In this study with an interpositional subperiosteal technique the connective tissue transplanted also demonstrated to influence the characteristics of the covering epithelium, enlarging the original area of KM.

Regarding the thickness of the keratinized tissue in Step 2, there was no statistically significant difference between the CTG and the CM. Cardaropoli [12] also found no differences between the connective tissue and the xenogeneic CM in relation to any clinical parameter, including KM thickness, in the treatment of Miller's class I and II gingival recessions using a surgical technique similar to that used in this study. In the maxilla, the entire palate is covered with KM, when the

implant placement procedures are performed it is relatively simple to move a band of KM to vestibular region at the emergence of the implants, however, in the lower jaw the lingual region is largely covered by mucosa alveolar, sometimes resulting in narrow band of residual KM. Implants that are not surrounded by KM are more prone to plaque buildup and soft tissue recession, even in patients who have sufficient oral hygiene and receive adequate periodontal therapy support [14]. To have a band ≥ 2mm, both vestibular and lingual to the emergence of the implants, the residual band should be ≥ 4mm, which does not always occur. In dental or implant regions where there is insufficient band of KM, this volume can be achieved by grafts procedures [11, 14]. Particularly in the posterior regions of edentulous mandibles, where reabsorption of the border leads to a reduction of vestibular depth and lack of KM, the apical displacement of the flap with an additional free gingival graft may be beneficial to facilitate adequate oral hygiene procedures and optimal control of the plate [14, 15]. However, free gingival grafts need access to a donor areas leading to greater morbidity. The autogenous graft of the subepithelial connective tissue of the palate used in an interpositional submucosal or subperiosteal manner is a therapeutic option for the areas with few KM, although it also needs access to a donor area, it presents a lower morbidity and a lower failure rate compared to free gingival graft [2, 3].

Also, in Step 2, the two types of grafts did not present a statistically significant difference in the number of fibroblasts per area, this histomorphometric evaluation of the fibroblasts in the grafted area represents the biocompatibility of the materials used. Lima [7] in their study also demonstrated the proliferation and viability of human gingival fibroblasts cultured in vitro on a CM. Another finding of our study was that there was no statistically significant difference in the counts of the native area fibroblasts compared to the GA, both for CTG and for the CM, with no clinical and histological signs of inflammatory process, but a proper cellular colonization.

In agreement with Rocchietta [5] in an animal model, no inflammatory adverse reactions were noticed in any specimen, resulting in optimal integration of the device, the CM was seen in place for the first 2 weeks, and it was completely replaced by healthy connective tissue within 30 days in the inlay position. In this human clinical study no inflammatory adverse reactions were, the CM was completely replaced by healthy connective tissue in Step 2 (2-3 months).

Future assessing of the grafted area by evaluating i*n vivo* vascular neoformation will help to provide information on tissue vitality and vascular and cellular responsiveness in cases of physical, chemical and microbial aggressions to the new tissue. Other materials for peri-implants soft tissue augmentation are commercially available and should be tested.

5. Conclusions
Within the limits of the study, clinical measurements for both grafted materials resulted in increased the width and thickness of KM while maintaining similar fibroblast counts for both GA and ANIG, demonstrating to be useful in peri-implant tissue augmentation. The CTG exhibited a statistically significant superiority in increasing the width of the KM, may be better indicated for areas with severe lack of KM (KM < 2mm).

6. Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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