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Distance of insertion points in a mattress suture from the wound margin for ideal primary closure in alveolar mucosa: an *in vitro* experimental study

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ABSTRACT

Purpose: This study was conducted to determine how the distance of the near insertion points in a vertical mattress suture from the wound margin influences the pattern of primary closure in an *in vitro* experimental model.
Methods: Pairs of 180 porcine gingival and alveolar mucosa samples were harvested from 90 pig jaws and fixed to a specially designed model. A vertical mattress suture was performed with the near insertion point at 3 different distances from the wound margin (1-, 3-, and 5-mm) on both the gingival and mucosal samples (6 groups; n=30 for each group). The margin discrepancy and the presence of epithelium between the wound margins were measured on histologic slides.
Results: The margin discrepancy decreased significantly as the near insertion point became closer to the wound margin both in mucosal tissue (0.241±0.169 mm, 0.945±0.497 mm, and 1.306±0.773 mm for the 1-, 3-, and 5-mm groups, respectively) and in gingival tissue (0.373±0.304 mm, 0.698±0.431 mm, and 0.713±0.691 mm, respectively). The frequency of complications of wound margin adaptation reduced as the distance of the near insertion point from the wound margin decreased both in the mucosal and gingival tissues.
Conclusions: Placing the near insertion point close to the wound margin enhances the precision of wound margin approximation/adaptation using a vertical mattress suture.

Keywords: Clinical protocol; Suture techniques; Wound closure techniques; Oral surgical procedures; Guided tissue regeneration

INTRODUCTION

The vertical mattress suture technique has frequently been recommended for various oral regenerative surgical procedures, such as in guided tissue regeneration [1], minimally invasive surgery [2], and guided bone regeneration [3,4]. This suture technique was initially introduced for dermal tissue based on the advantages of precise adaptation, an everting

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Author Contributions
Conceptualization: Jung-Seok Lee; Formal analysis: Jung-Seok Lee; Methodology: Won-Ho Lee; Project administration: Jung-Seok Lee; Supervision: Jung-Seok Lee; Writing - original draft: Won-Ho Lee, Jung-Seok Lee; Writing - review & editing: Ulrike Kuchler, Jae-Kook Cha, Andreas Stavropoulos.

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

wound margin, and movement of the tension away from the margin [5]. The vertical mattress suture is also used in dentistry for the same purposes; that is, resisting muscle pulling, everting the wound margins, and firmly adapting the tissue flaps to underlying structures such as bones, regenerative membranes, and implants [6,7]. However, although some characteristics differ between the oral mucosa and dermal tissue (thin and ridged versus thick and flat) [8], there are no clear guidelines for the use of the vertical mattress suture on the oral mucosa.

Wound healing depends on early vascularization from 2 opposing tissues, and the vertical mattress suture aims at enhancing the adaptation between vessel-abundant connective tissues from the superficial to deep layers. However, even though the mattress suture technique has been applied in some cases, incomplete wound healing such as dehiscence can occur following alveolar augmentation [9-11]. The presence of epithelium between the connective tissues of wound margins is a causal risk factor for wound dehiscence, in addition to the flap thickness and tension [7,12]. In this context, properly controlling the insertion points of the suture appears critical for the precise approximation of the wound margins from a mechanical standpoint [13,14].

In the conventional protocol of the vertical mattress suture for dermal tissue, the far-far insertion points are recommended to be located 4 to 8 mm from the wound margin, and the near-near insertion points at 1 to 2 mm [15]. However, for the purpose of enhancing wound margin adaptation and promoting wound healing, closer near-near insertion points should be applied. In addition, the farther insertion points in a mattress suture cannot be used to apply force in an appropriate direction for ensuring margin adaptation by tightening the suture. Further, it has been suggested that the near-near insertion point should be as superficial as possible [5], and that when the near-near insertion point is far from the wound margin, healing is delayed and/or compromised due to an "eversion-inversion phenomenon" at the 2 wound margins that remain unsupported [16]. However, there has been no systematic assessment of how the distance of the insertion point from the wound margin in the vertical mattress suture influences the pattern of primary wound closure in gingival and oral mucosal incisional wounds.

This study aimed to determine how the distance of the insertion point closest to the wound margin in a vertical mattress suture influences the pattern of primary wound closure using an *in vitro* experimental model mimicking the alveolar ridge crest.

MATERIALS AND METHODS

Study design

This *in vitro* study was performed using alveolar mucosa and gingiva from pig jaws as described in a previous study that evaluated suture tension [17]. The harvested porcine mucosa and gingiva were fixed on a specially designed model fabricated by replicating the dental cast of a partially edentulous maxilla using silicone putty (HySil Putty, Osstem, Seoul, Korea) (Figure 1), and suturing was performed. Six experimental groups were formed according to the distance of the near insertion point from the wound margin and the type of oral mucosa: 1-, 3-, and 5-mm in the mucosa and gingival flaps.

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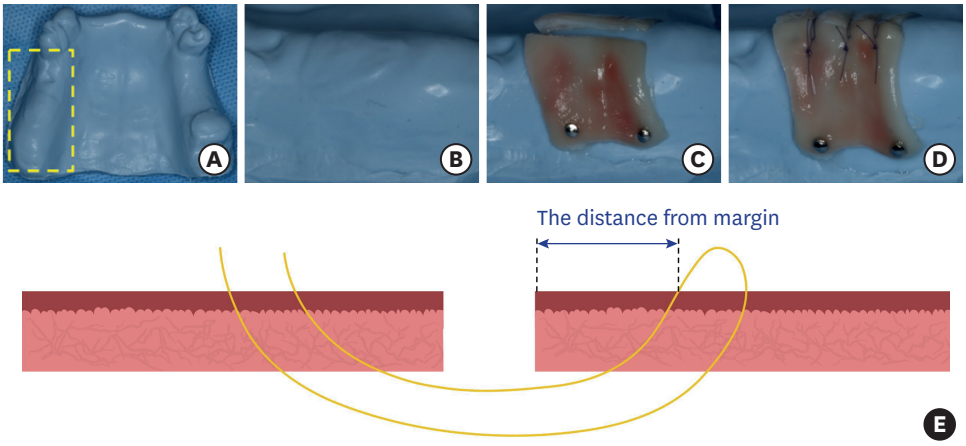

Figure 1. Study design and experimental procedures. (A) To mimic the shape of the alveolar ridge crest, a specially designed experimental model was fabricated using silicone putty by duplicating the dental cast of a partially edentulous maxilla. (B) To reproduce the clinical setting, the ridge-shaped edentulous area of the silicone model was used in suture experiments. (C) One pair of harvested tissue samples (standardized dimensions: 12 mm × 12 mm) from porcine gingiva and mucosa were fixed to the silicone model using pins, with 1-mm of overlap between the wound margins. (D) Three modified vertical mattress sutures were performed on the fixed tissue samples at intervals of 3-mm. The near insertion point of the vertical mattress suture was located 1-, 3-, or 5-mm from the wound margin depending on the group assignment, and the far insertion point was located 3-mm from the near insertion point. After the sutures were completed, all samples showed precise wound margin adaptation and tight primary closure in clinical observations. (E) Schematic of the experimental model. The distance of the near insertion point from the wound margin was the main variable. Three groups of mucosal and gingival tissue samples were produced according to the position of the near insertion point.

Experimental model

This study retrieved 180 alveolar mucosa samples and 180 gingival samples from 90 fresh lower pig jaws. One pair of standardized mucosa samples (12 mm × 12 mm) obtained from the floor of the mouth (nonkeratinized tissue with a thickness of 1–1.5 mm) and 1 pair of gingival samples with the same dimensions obtained from the alveolar ridge (keratinized tissue with a thickness of 2–4 mm) were harvested from each pig jaw using a surgical scalpel and a periosteal elevator. The tissue samples were kept moist with wet gauzes soaked with saline and stored in a refrigerator until suturing in order to prevent desiccation and tissue degeneration. To mimic the clinical setting for suturing, 1 pair of harvested tissue samples (mucosa or gingiva from the same pig jaw) was fixed (tacked) to a silicone model using metal pins at a position that allowed 1-mm of overlap between the wound margins. Finally, a total of 90 mucosal and 90 gingival experimental models were prepared and divided into each of 3 experimental groups according to the distance of the near insertion point from the wound margin and the type of tissue.

Experimental procedures

Three vertical mattress sutures were performed in 1 prepared experimental model with intervals of 3-mm. One expert (W.H.L.) performed the vertical mattress sutures under professional supervision (J.S.L.). The suture was started using far-to-far insertion followed by near-to-near insertion between the tissue flaps on the buccal and palatal sides of the prepared model, using a monofilament suture (Monosyn 6-0, B.BraunAesculap, Tuttlingen, Germany). The near insertion points were positioned according to the group allocation, and the far insertion point was located 3-mm from the near insertion point. The knot was formed on the buccal side after passing through the loop between the near and far points on the palatal side (modified vertical mattress suture technique). After placing the sutures, all samples showed tight primary closure and there was no discrepancy between the flaps in clinical observations. The sample (including the model) was fixed with 10% neutral buffered formalin.

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Histologic preparation

After 7 days of fixation, the specimens were mechanically fixed using an additional fixation pin at the center of the sample in order to minimize deformation during gross sectioning, and the specimens containing sutures were sectioned at the most-central region. The sectioned specimens were placed in tissue processing cassettes and fixed in buffered formalin for 2 additional days. The silicone putty was then carefully removed, and the residual specimen was dehydrated by immersing it in a series of ethanol solutions. The specimens were embedded in paraffin and cut along the longitudinal axis immediately adjacent to the central suture to a thickness of 4 μm using a microtome (RM2255, Leica, Nussloch, Germany). The sections were stained with hematoxylin-eosin, and histologic slides were digitally scanned and converted into digital image files for the histologic analysis.

Margin discrepancy between wound margins

The discrepancy between the 2 wound margins (i.e., the vertical difference between the tops of the epithelium in the 2 wound margins) was measured on histologic slides (Figure 2) using computer software (CaseViewer2.0, 3DHisTech, Budapest, Hungary) to evaluate the accuracy of primary closure according to the distance of the near insertion points. The maximum value measured on 3 histologic slides of the same experimental sample was considered to be the representative value of that sample.

Histologic observations of complicated primary closure

Considering that the purpose of a suture is to make 2 wound margins meet precisely, the occurrence of certain complications in primary closure is a risk factor for delayed healing. The frequencies of the following complications were counted to evaluate the pattern of primary closure (Figure 3):

1. Overlapping, where the epithelium of one of the flaps rolls down the other one, resulting in contact of the epithelium with the connective-tissue layer.

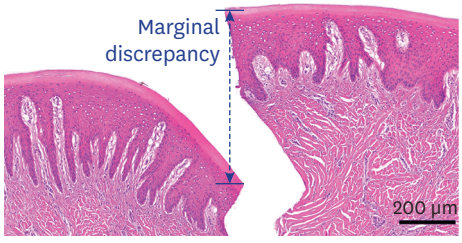

Figure 2. Histologic measurements of the margin discrepancy. The vertical distance between the tops of the epithelium of the 2 wound margins was measured on histologic slides.

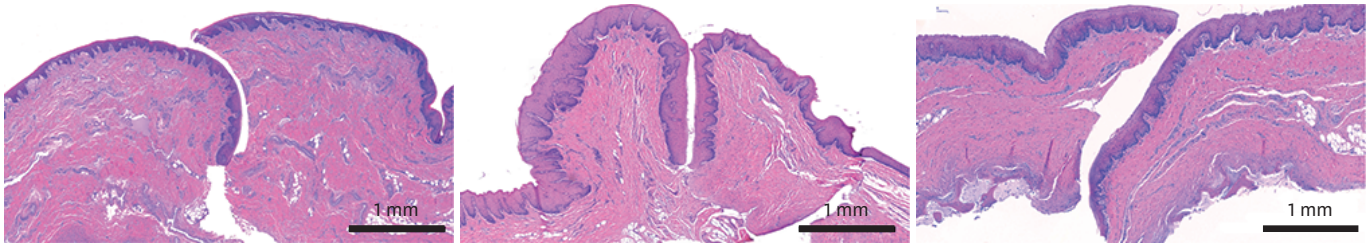

Figure 3. Classification of complications according to the flap adaptation morphologies. Three types of complications were considered in this study: 1) overlapping, where the epithelium of one flap rolls down the other one; 2) folding, where both wound margins are folded such that the outer epithelial surfaces contact each other; and 3) sliding, where one flap slides completely under the connective tissue of the opposing flap.

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2. Folding, where both wound margins are folded such that the outer surfaces of the epithelium are in contact with each other.
3. Sliding, where one flap slides completely under the connective tissue of the opposing flap.

Statistical analysis

A required sample size of 30 samples per group was calculated using R software (R 3.6.3, The R Foundation, Vienna, Austria) with the margin discrepancy as the primary outcome, for 1-way analysis of variance with a 2-sided alpha level of 5%, a statistical power of 95%, and an effect size of 0.4 [18]. The statistical analysis was performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). The mean and standard deviation values of the margin discrepancy were calculated. Whether the data conformed to a normal distribution was evaluated using the Kolmogorov-Smirnov test, with either 1-way analysis of variance or the Kruskal-Wallis test then applied accordingly. Either the Bonferroni or Mann-Whitney *post hoc* test was also applied. The frequencies of complications were compared between experimental groups using the Pearson χ^2 test and the Fisher exact test. The criterion for statistical significance was set at $P<0.05$.

RESULTS

All samples showed tight primary closure of the flaps after suturing in clinical inspections. However, the histologic results revealed various types of contact between the 2 flaps, including complications such as a dislocated apposition of each wound margin and epithelium being caught between the 2 wound margins (overlapping, folding, and sliding; Figure 3).

Measurements of the margin discrepancy

The measured margin discrepancies are presented in Table 1 and Figure 4. The margin discrepancies in the mucosal samples were 0.241±0.169 mm, 0.945±0.497 mm, and 1.306±0.773 mm for the 1-, 3-, and 5-mm groups, respectively; the corresponding values in the gingival samples were 0.373±0.304 mm, 0.698±0.431 mm, and 0.713±0.691 mm. Multiple comparisons revealed that the 1-mm group showed a statistically significant difference compared to 3-mm and 5-mm groups in the mucosal samples, and to the 3-mm group in gingival samples.

Frequency of complications

The results for the frequency of complications are presented in Table 2. In general, complications were more frequent in samples of the oral mucosa than in the gingival samples. The most frequent complication in all of the samples was overlapping (53.62% and 85.37% of the mucosal and gingival samples, respectively), followed by sliding (36.23% and 14.63%) and

Table 1. Marginal discrepancy measurement results in gingival and mucosal tissue

Marginal discrepancy (mm)	1-mm (n=30)	3-mm (n=30)	5-mm (n=30)	*P*-value[f]
Gingival tissue	0.373±0.304[a,b]	0.698±0.431[a]	0.713±0.691[b]	0.028
Mucosal tissue	0.241±0.169[c,d]	0.945±0.497[c,e]	1.306±0.773[d,e]	<0.001

Data are presented as mean±standard deviation.
[a]Significant difference ($P=0.042$) between the 1-mm group and the 3-mm group in gingival tissue; [b]Significant difference ($P=0.030$) between the 1-mm group and the 5-mm group in gingival tissue; [c]Significant difference ($P<0.001$) between the 1-mm group and the 3-mm group in mucosal tissue; [d]Significant difference ($P<0.001$) between the 1-mm group and the 5-mm group in mucosal tissue; [e]Significant difference ($P=0.034$) between the 3-mm group and the 5-mm group in mucosal tissue; [f]One-way analysis of variance.

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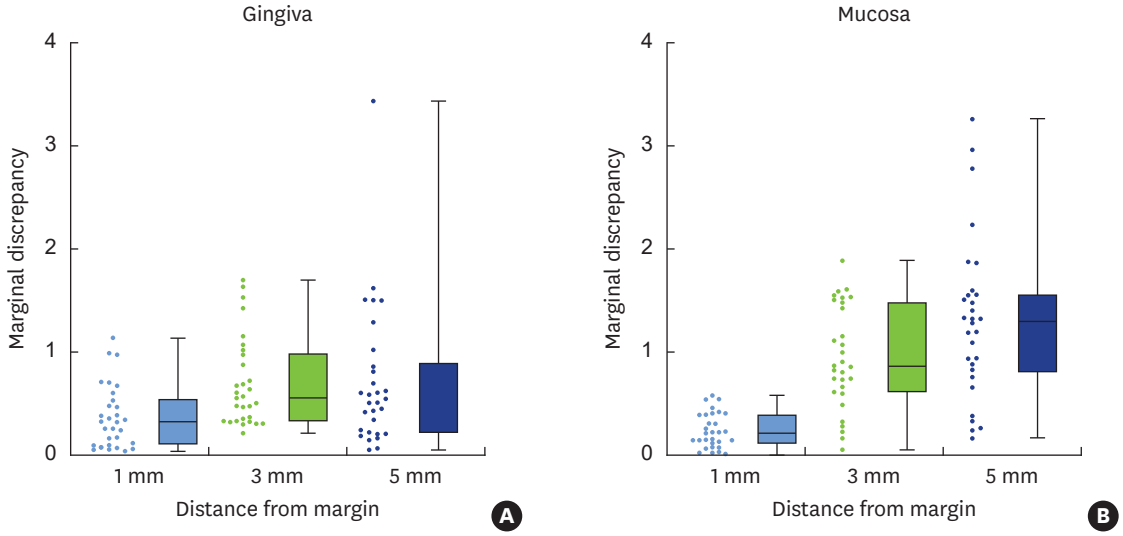

Figure 4. Histologic measurements of the margin discrepancies in the gingival and mucosal tissues. Each box plot indicates the median, first and third quartiles, and maximum and minimum values of the margin discrepancy. The scatter plot shows the distribution of the margin-discrepancy values within each group. The margin discrepancy both in mucosal and in gingival tissues tended to decrease when the near insertion point was closer to the wound margin.

folding (10.14% and 0%). The total frequency of complications was significantly lower in the 1-mm group (43.33%) than in the 3-mm (93.33%) and 5-mm (93.33%) groups for the mucosal samples (P=0.005). The frequency of sliding in mucosal samples was also significantly lower in the 1-mm group (0%) than in both the 3-mm (33.33%) and 5-mm (50.00%) groups (P=0.005). Overlapping and folding occurred at similar frequencies regardless of the distance of the near insertion point from the wound margin. The frequency of complications in gingival samples also showed a non-statistically significant tendency to increase as the near insertion point moved farther from the wound margin (36.67%, 43.33%, and 56.67% for the 1-, 3-, and 5-mm groups, respectively; P>0.05). Only sliding in gingival samples occurred at a significantly higher rate in the 5-mm group, and it did not occur in the 1-mm group.

Table 2. Frequency of complications in the gingival and mucosal tissue

Complication	Group			Proportion
	1-mm	3-mm	5-mm	
Mucosa[a]				
Overlap	10/30 (33.33)	15/30 (50.00)	12/30 (40.00)	53.623
Folding	3/30 (10.00)	3/30 (10.00)	1/30 (3.33)	10.145
Sliding	0/30 (0)	10/30 (33.33)	15/30 (50.00)	36.232
Total	13/30 (43.33)	28/30 (93.33)	28/30 (93.33)	-
Gingiva				
Overlap	11/30 (36.67)	12/30 (40.00)	12/30 (40.00)	85.366
Folding	0/30 (0)	0/30 (0)	0/30 (0)	0
Sliding	0/30 (0)	1/30 (3.33)	5/30 (16.67)	14.634
Total	11/30 (36.67)	13/30 (43.33)	17/30 (56.67)	-
Overall[a]				
Overlap	21/60 (35.00)	27/60 (45.00)	24/60 (40.00)	65.455
Folding	3/60 (5.00)	3/60 (5.00)	1/60 (1.67)	6.363
Sliding	0/60 (0)	11/60 (18.33)	20/60 (33.33)	28.182
Total	24/60 (40.00)	41/60 (68.33)	45/60 (75.00)	-

Values are presented as number (%).
[a]Statistically significant difference between the 1-mm group and the 3- and 5-mm groups (P<0.05).

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DISCUSSION

Suturing is usually the last step in oral surgery and critically affects the clinical success of procedures. Precise approximation of the wound margins is one of the requirements for an ideal suture [13], and the vertical mattress suture is one of the most widely applied techniques with this aim in the fields of periodontal and implant surgery [19]. However, there is little scientific evidence in the literature that would inform a guideline for the clinical protocol of suturing, especially regarding how the details of each step influence the outcome.

The present *in vitro* study is the first to evaluate how the primary closure pattern varies with the distance of the near insertion point from the wound margin for vertical mattress sutures in mucosal and gingival tissues (Figure 5). Two main findings were obtained: 1) the closest near insertion point of the vertical mattress suture (i.e., 1-mm from the wound margin) reduced the discrepancy between the 2 wound margins both in mucosal and in gingival tissues, with the difference being statistically significant in mucosal tissue, and 2) the frequency of complications (epithelium being caught between the flaps) was significantly higher in specimens with farther near insertion points (3- and 5-mm) than in those with the closest near insertion point (1-mm) both in mucosal and in gingival tissues.

Wound healing includes inflammatory, proliferative, and remodeling phases [20]. The first 2 phases are delayed in cases of secondary-intention healing (e.g., with wound dehiscence). In contrast, in primary-intention healing, the precisely approximated wound margins share the same structure of epithelium and connective tissue layers as well as small vessels, and wound healing is promoted by minimizing the inflammatory and proliferative tissue phases. However, improper suturing can result in an unsupported or gapped superficial wound margin and/or a discrepancy between the wound margins, which will negatively influence healing and

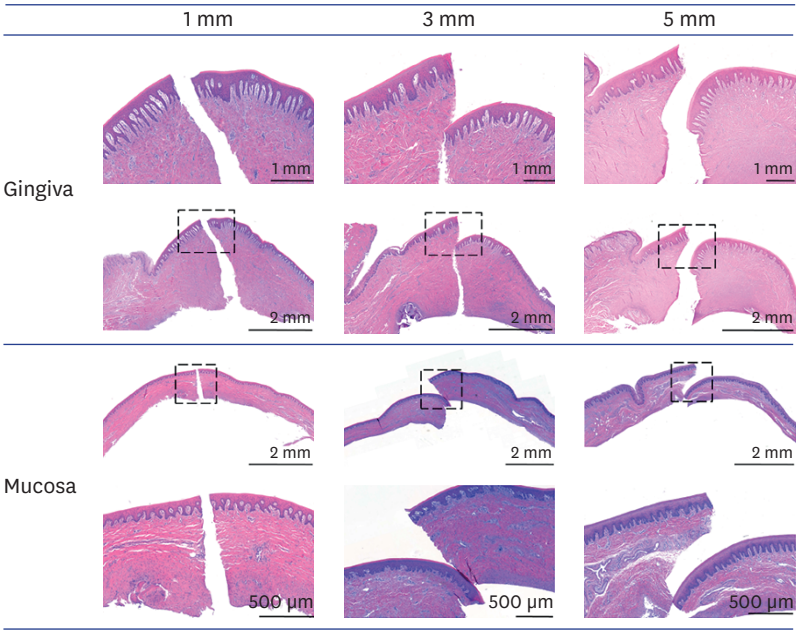

Figure 5. Representative histologic slides in each group of mucosal and gingival tissues. The dotted area in each low-magnification histologic slide is displayed in the corresponding high-magnification slide. The flap approximation was more precise both in mucosal and in gingival tissues when the near insertion point was closer to the wound margin.

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may lead to scar formation [16]. A previous clinical study of dermal tissue suturing found that an additional interrupted suture could enhance the approximation of the superficial wound margins and reduce scar formation compared to a single vertical mattress suture in the deeper layer of a wound [21]. The vertical mattress suture was originally devised by Mario Donati to enhance the approximation of wound margins and move the tension from the wound margin to the intermediate flap region between the near and far insertion points [5]. In oral mucosa suturing for augmentation surgery, a modified version of mattress suturing was also introduced to increase the precise approximation of the superficial wound margins [19].

The present study found that the precision of the approximation of the flaps in mattress sutures was significantly enhanced when the near insertion point was closer to the wound margin. This corresponds to the methodology of the originally devised mattress suture technique for dermal tissue, for which it was suggested that the near insertion point be placed as superficially as possible [5]. Another previous report described a complicated situation involving the "eversion-inversion phenomenon," in which the superficial region was unsupported and healing was delayed for the farther near insertion point in the mattress suture [16]. When performing this suture technique on the crest of the alveolar ridge, this pattern can be exaggerated due to the flap tension acting in the apical direction, especially in augmentation surgery.

The optimal distance of the near insertion point should be separately considered along with the optimal bite size of the single interrupted suture. Some experts have recommended that the bite size should never be less than 1–2 mm, due to the risk of tearing the mucosal wound margin [22]. In contrast, in the mattress suture, the tension is concentrated in the area between the near and far insertion points rather than around the wound margin; therefore, using closer near insertion points might enhance the precision of the approximation between the flaps without inducing excessive stress at the wound margin.

The presence of epithelial tissue between 2 wound margins can delay the healing process. Incised wound margins share the continuous structures of the epithelium, connective tissue, and vessels, and these can be unified with minimal inflammatory and proliferative responses. However, wound healing can be delayed by degradation of a squeezed epithelial layer, which extends the initial healing phases of inflammation. In particular, if the epithelium slides under the opposing flap, this may induce complete separation of each flap margin and limited wound healing, and it can be the beginning of wound dehiscence or membrane exposure. A previous clinical study of lateral ridge augmentation found that 70% of wound dehiscence occurred in the mandibular region, which showed more mucosal tissue and less gingival tissue than the maxilla [23]. The present study found that a significantly increased rate of overall (epithelium-related) complications and especially of sliding epithelium can produce the aforementioned clinical results. However, the occurrence of complications (even in mucosal tissue) was significantly reduced when the near insertion point was closer to the wound margin (1-mm). Therefore, the guideline of a closer near insertion point should be emphasized, especially when suturing mucosal tissues.

The present study was performed *in vitro* using harvested mucosal and gingival samples in an alveolar ridge-shaped model mimicking the clinical setting, which could have resulted in critical limitations due to differences between vital and nonvital tissues and between human and porcine tissues. Many other factors such as flap advancement may also affect discrepancies or other complications in primary closure. Therefore, the present results need to be confirmed in future studies with other designs and clinical settings.

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Notwithstanding the limitations of this study, the present results clearly reveal tendencies for an increasing discrepancy of the wound margin and an increased rate of epithelium-related complications when the near insertion points were further from the wound margin (3- and 5-mm) compared to the closest near insertion point (1-mm). These findings may represent scientific evidence for the guideline of using closer near insertion points in the mattress suture technique.

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