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Original Article

Coronal flap advancement achieved by butterfly and sling sutures in a sheep cadaver tunneling model: An ex vivo randomized paired experimental study

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Abstract

Background. Suturing configuration may influence coronal flap positioning during root coverage procedures. This study compared the immediate coronal flap advancement achieved by butterfly and sling sutures in a standardized sheep cadaver tunneling model.

Methods. This ex vivo, randomized, paired study included six sheep mandibles, with 12 experimental sites. After preparing the tunneling flap and standardizing baseline tension to 0.60 N, we placed butterfly and sling sutures at each site in a randomized sequence. Coronal flap advancement was measured using a digital caliper. The primary comparison was performed using a paired-samples t-test, with 95% confidence intervals (CIs) and a Hedges-corrected standardized effect size. Period, sequence, and mandible-level sensitivity analyses were also performed.

Results. Mean coronal flap advancement was 3.48 ± 0.64 mm with the butterfly suture and 2.85 ± 0.69 mm with the sling suture. The butterfly suture achieved significantly greater advancement, with a mean paired difference of 0.63 mm (95% CI: 0.41–0.85 mm; $P < 0.001$). The Hedges-corrected standardized effect size was 1.79 (95% CI: 0.86–2.70). No statistically significant period effect was detected (mean difference: -0.08 mm; 95% CI: -0.55 to 0.39 mm; $P = 0.719$), and the butterfly–sling treatment effect did not differ significantly according to application sequence (mean sequence difference: 0.16 mm; 95% CI: -0.37 to 0.68 mm; $P = 0.480$). In the mandible-level sensitivity analysis, the mean butterfly–sling difference remained 0.63 mm (95% CI: 0.38–0.88 mm; $P = 0.001$).

Conclusions. Under controlled ex vivo conditions, the butterfly suture produced greater immediate coronal flap advancement than the sling suture. The clinical significance of the 0.63-mm difference remains uncertain and requires investigation in clinical studies.

Graphical Abstract

Butterfly vs. Sling sutures

Coronal advancement; ex vivo tunneling model

6 sheep mandibles: 12 sites, with both sutures at each site.
Randomized application order



Greater immediate advancement with Butterfly
Clinical significance remains uncertain.

Key words: Butterfly suture, gingival recession, periodontal plastic surgery, root coverage, tunneling technique.

Introduction

Gingival recession (GR), defined as apical displacement of the gingival margin relative to the cemento-enamel junction, is a common mucogingival condition that may cause esthetic concerns, dentin hypersensitivity, root caries, and difficulty controlling plaque.^{1,2} Recent epidemiological studies continue to demonstrate a high prevalence of gingival recession among adult populations, emphasizing its clinical relevance in contemporary periodontal practice.^{3,4} The management of GR has therefore become an important component of periodontal plastic surgery, with root coverage procedures aiming not only to restore soft tissue esthetics but also to improve patient comfort and maintain long-term periodontal health.⁵ Among the various approaches available, the use of a connective tissue graft (CTG) in combination with coronally advanced soft tissue procedures remains the most predictable treatment modality for achieving root coverage.^{6,7}

In recent years, tunneling techniques have gained considerable popularity because they preserve papillary integrity, maintain vascular supply, minimize surgical trauma, and provide favorable esthetic outcomes.⁸ Among the various root coverage approaches currently available, tunnel techniques combined with connective tissue grafts have demonstrated predictable clinical outcomes and high levels of root coverage in the treatment of both localized and multiple gingival

recessions, making them a widely accepted treatment option in contemporary periodontal plastic surgery.^{9,10}

Successful root coverage outcomes depend not only on flap mobilization and graft integration but also on achieving stable coronal positioning of the gingivopapillary complex, since flap stabilization and adaptation to the tooth surface are considered critical determinants of wound healing and treatment success.¹¹ By protecting the blood clot and limiting flap micromotion during the early healing phase, flap stability contributes directly to favorable tissue integration.¹² Consequently, achieving both stable flap fixation and adequate coronal advancement has become a major objective in the development of contemporary root coverage procedures and associated suturing protocols.^{13,14}

To enhance graft and flap fixation and facilitate coronal advancement in tunnel procedures, several suspensory and anchoring suturing approaches have been proposed, including the subpapillary continuous sling suture, suspensory sutures anchored to interdental contact points, anchored vertical and horizontal mattress sutures, double-crossed sutures, reverse-V sutures, and other modified sling-based configurations.^{13–18} Despite differences in design and anchorage mechanisms, these techniques share the common objective of achieving stable coronal flap positioning, enhancing graft–flap adaptation, and minimizing soft tissue micromotion during healing.

Building upon these concepts, the butterfly suture was recently introduced as a modified anchoring technique designed to simultaneously stabilize both the interproximal and midfacial portions of the buccogingival complex.¹⁹ Unlike many existing suspensory sutures that primarily rely on interdental anchorage, the butterfly suture incorporates diagonal anchoring around adjacent contact points to provide additional support to the midfacial aspect of the flap. This design was proposed to improve coronal fixation, enhance adaptation between the flap, graft, and root surface, and reduce flap micromotion during healing. Initial clinical reports demonstrated encouraging outcomes; however, these observations were derived from uncontrolled clinical cases.¹⁹

However, the specific contribution of the butterfly suturing design to coronal flap displacement could not be determined under clinical conditions, and its effect on immediate coronal advancement has not been objectively quantified. Furthermore, no experimental study has directly compared the ability of the butterfly and sling sutures to achieve immediate coronal flap advancement under controlled conditions. Therefore, this *ex vivo* study aimed to compare the amount of coronal flap advancement achieved by butterfly and sling sutures in a standardized sheep cadaver tunneling model. The null hypothesis was that the two suturing techniques would not differ significantly in coronal flap advancement.

Methods

Study Design

This *ex vivo* randomized paired experimental study was conducted to compare the amount of coronal flap advancement achieved using butterfly and sling suturing techniques in a tunneling model. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1404.033).

Six fresh sheep cadaver mandibles were included in the study. Suitable premolar teeth were selected on both sides of each mandible, resulting in 12 experimental sites. Suitable premolar sites were included if they exhibited intact soft tissues, absence of visible hard tissue defects, and adequate access for tunneling, suturing, and measurement procedures. Sites presenting with

fractures, extensive caries, soft tissue damage, or anatomical conditions that could interfere with flap preparation, tension standardization, suturing, or outcome assessment were excluded. The experimental site was considered the unit of analysis for the primary paired comparison, with each mandible contributing two experimental sites. Each site received both suturing techniques, allowing direct within-site comparison. Within each mandible, the two experimental sites were assigned to opposite application sequences, with one site receiving the butterfly suture first and the other receiving the sling suture first; sequence allocation to the sites was determined using computer-generated randomization.

Sample Size Considerations

Because no prior controlled biomechanical study directly comparing butterfly and sling sutures was available to provide reliable estimates for an effect size-based sample-size calculation, no formal power calculation was performed, and the present study was designed as an exploratory investigation. Six sheep mandibles were included, providing 12 experimental sites, with both suturing techniques evaluated at each site.

Experimental Site Preparation and Tension Standardization

At each experimental site, an intrasulcular incision was performed around the selected premolar tooth, and a tunneling flap was prepared using tunneling instruments (Hu-Friedy, Chicago, IL, USA) while preserving the interdental papillae. Soft tissue release was performed to facilitate passive coronal advancement of the flap.

To standardize baseline flap tension before suturing, a universal testing machine (UTM) (SANTAM, Iran) equipped with a load cell (DBBP-50; Bongshin, Korea) with a resolution of 0.01 N was used. A temporary mattress suture was placed at the midfacial aspect of the flap margin and connected to the testing device. The flap was pulled coronally to a predefined reference position located 3 mm coronal to the initial gingival margin, and soft tissue release was adjusted until a tensile force of 0.60 N was required to reach this position. The 0.60 N reference force was selected as a predefined experimental standardization parameter rather than a clinically recommended threshold; it was used solely to establish comparable baseline mechanical conditions across experimental sites. This calibration procedure was performed for all experimental sites before application of the test sutures.

Suturing Procedures

After tension standardization, we placed both butterfly and sling sutures at each experimental site using 5-0 nylon sutures (Hu-Friedy, Chicago, IL, USA). At six experimental sites, the butterfly suture was applied first, followed by the sling suture after removal of the initial suture. At the remaining six sites, the sling suture was applied first, followed by the butterfly suture.

The butterfly suture was performed according to the technique originally described by Khojaste et al.,¹⁹ whereas the sling suture was performed using a conventional suspensory sling configuration. All suturing procedures were performed by the same experienced periodontist to minimize operator-related variability.

Outcome Assessment

The primary outcome was the amount of coronal flap advancement achieved by each suturing technique. Before suturing, the initial position of the gingival margin was marked using a fine-tip marker. After applying and tightening the assigned suture, the new gingival margin position was

marked again. The distance between the two markings was measured using a digital caliper (Inchsize, China) with a resolution of 0.01 mm and recorded as the amount of coronal flap advancement. All measurements were performed by the same experienced periodontist. Blinding of the operator and outcome assessor to the suturing technique was not feasible because the butterfly and sling suture architectures were visibly distinct during placement and measurement. Each measurement was immediately repeated once as a procedural consistency check. No intraclass correlation coefficient (ICC) or other formal intra-examiner reliability or measurement-error analysis was performed. After measurement of the first suturing technique, the suture was removed. After removing the first suture, the flap was returned to its original marked baseline position before applying the second suturing technique. The same outcome-assessment procedure was then repeated at the same experimental site using the alternative suturing technique. Figure 1 shows representative images of the experimental setup, suturing procedures, and outcome assessment.

Statistical Analysis

Statistical analyses were performed using SPSS 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were reported as mean $\pm$ standard deviation (SD), median with interquartile range (IQR), and minimum–maximum values. Normality of the relevant difference scores was assessed using the Shapiro–Wilk test.

The primary comparison of coronal flap advancement between the butterfly and sling sutures was performed using a paired-samples t-test because both techniques were evaluated at each experimental site. Mean paired differences and their 95% confidence intervals (CI) were calculated. For the paired comparison, the standardized effect size was calculated using the SD of the paired differences as the standardizer. Cohen's d_z was calculated as the mean paired difference divided by the SD of the paired differences. The Hedges-corrected standardized effect size and its 95% CI were subsequently obtained using the paired-samples effect-size procedure in SPSS, with the SD of the paired differences specified as the standardizer and the software's small-sample bias correction applied.

Additional analyses were performed to evaluate potential effects associated with sequential testing. A period effect was assessed by calculating the difference between the second and first measurements at each experimental site and testing the mean difference against zero using a one-sample t-test. To assess whether the butterfly–sling treatment effect depended on the order of application, the butterfly–sling difference was calculated for each site. Within each mandible, the treatment effect at the butterfly-first site was compared with that at the sling-first site, and the resulting intra-mandible sequence differences were tested against zero using a one-sample t-test. Because two experimental sites were obtained from each mandible, a mandible-level sensitivity analysis was performed to assess the robustness of the primary finding to possible intra-mandible dependence. The butterfly–sling differences from the two sites belonging to each mandible were averaged to obtain one treatment-effect estimate per mandible, and these six mandible-level differences were tested against zero using a one-sample t-test. No formal multivariable linear mixed-effects model was fitted. All statistical tests were two-sided, and statistical significance was set at $P < 0.05$.

Results

Twelve experimental sites obtained from six sheep mandibles were included in the analysis. The distribution of paired differences in coronal flap advancement was compatible with normality (Shapiro–Wilk $W=0.943$, $P=0.538$).

The mean coronal advancement with the sling suture was 2.85 ± 0.69 mm, whereas the butterfly suture achieved 3.48 ± 0.64 mm. Median (IQR) values were 2.84 (1.01) mm and 3.51 (0.86) mm for the sling and butterfly sutures, respectively (Table 1).

Paired comparison demonstrated significantly greater coronal advancement with the butterfly suture than with the sling suture. The mean paired difference was 0.63 mm (95% CI: 0.41–0.85 mm, $P<0.001$). The Hedges-corrected standardized effect size was 1.79 (95% CI: 0.86–2.70), indicating a large standardized difference between the two techniques under the study's experimental conditions (Table 2).

Additional analyses were performed to evaluate potential effects related to sequential testing. For the period-effect analysis, the distribution of the second-minus-first measurement differences was compatible with normality (Shapiro–Wilk $W=0.918$, $P=0.271$). The mean difference between the second and first measurements was -0.08 mm (95% CI: -0.55 to 0.39 mm), and no statistically significant period effect was detected ($t(11)=-0.37$, $P=0.719$).

The intra-mandible sequence differences were also compatible with normality (Shapiro–Wilk ($W=0.970$, $P=0.892$)). On average, the butterfly–sling treatment effect was 0.16 mm greater when the butterfly suture was applied first than when the sling suture was applied first; however, this difference was not statistically significant (95% CI: -0.37 to 0.68 mm; $t(5)=0.76$, $P=0.480$).

Finally, a mandible-level sensitivity analysis was performed by averaging the butterfly–sling differences from the two sites in each mandible. The resulting mandible-level differences were compatible with normality (Shapiro–Wilk $W=0.870$, $P=0.227$). The mean mandible-level butterfly–sling difference remained 0.63 mm (95% CI: 0.38–0.88 mm) and was statistically significant ($t(5)=6.52$, $P=0.001$) (Table 3).

Discussion

This study compared immediate coronal flap advancement with butterfly and sling sutures in a standardized sheep cadaver tunneling model. The butterfly suture produced significantly greater coronal advancement than the sling suture, with a mean paired difference of 0.63 mm (95% CI: 0.41–0.85 mm). The Hedges-corrected standardized effect size was large ($g = 1.79$; 95% CI: 0.86–2.70), indicating a marked difference between the two suturing techniques under the controlled experimental conditions of the present study. Additional analyses did not detect significant period or sequence effects, and the mandible-level sensitivity analysis yielded a consistent statistically significant difference. Accordingly, the null hypothesis of no difference between the two techniques was rejected for the outcome of immediate coronal flap advancement.

One possible explanation for the greater coronal advancement achieved with the butterfly suture is its anchoring configuration and traction force distribution. Unlike conventional sling sutures, which primarily rely on interdental anchorage to position the flap coronally, the butterfly suture was designed to simultaneously engage both the interproximal and midfacial portions of the buccogingival complex. This configuration may distribute tensile forces more broadly across the flap and facilitate greater coronal displacement during suture tightening. Furthermore, the butterfly suture's diagonal anchoring pathway may generate combined coronal and horizontal traction

forces that facilitate coronal displacement during suture tightening.¹⁹ Nevertheless, these biomechanical mechanisms were not directly measured in the present study, and further experimental work incorporating direct force-vector analysis, flap stability testing, and micromotion assessment is needed to clarify the mechanical basis of the observed difference.

The greater immediate coronal advancement observed with the butterfly suture should also be considered in relation to tissue vascularity. Although its configuration may distribute coronal traction across a broader tissue area, excessive horizontal compression of the flap against the underlying root or bone could compromise marginal microvascular perfusion *in vivo*.^{19,20} This possibility may warrant particular caution in thin periodontal phenotypes, although perfusion and phenotype-specific effects could not be assessed in the present cadaveric model. Therefore, greater mechanical advancement should be balanced against preservation of flap perfusion and avoidance of tissue ischemia.

Although coronal flap advancement is an important component of root coverage procedures, the clinical significance of the 0.63-mm difference observed in the present *ex vivo* model remains uncertain. Pini Prato et al.²¹ reported that the postsurgical position of the gingival margin relative to the cemento-enamel junction significantly influences the likelihood of complete root coverage, supporting the importance of adequate coronal positioning at the time of surgery. Consequently, even modest differences in flap advancement at the time of suturing may influence the initial soft-tissue position and potentially affect subsequent clinical outcomes. However, the present study evaluated only immediate mechanical displacement in cadaveric tissue and could not reproduce dynamic muscle pull, displacement by tongue movements, postoperative swelling or shrinkage, vascular perfusion, wound contraction, graft integration, or tissue remodeling. Therefore, although the 0.63-mm difference was statistically significant ($P < 0.001$), it cannot be directly extrapolated to higher rates of complete root coverage or clinical treatment success in living patients. Rather, it reflects a measurable difference in immediate flap positioning under standardized experimental conditions, and its clinical relevance requires confirmation in prospective clinical studies.

Suturing techniques for root coverage procedures have evolved from simple suspensory approaches to more complex anchoring configurations designed to improve flap positioning and stabilization. Early sling-based techniques relied primarily on interdental anchorage to maintain the flap in a coronal position, whereas later modifications added anchorage points and altered force vectors to improve control of the buccogingival complex.^{13–18,22} Examples include anchored mattress sutures, double-crossed sutures, reverse-V sutures, and other modified suspensory designs. Collectively, these developments reflect continued efforts to optimize the mechanical interaction between the suture and flap. In a cadaveric study, Tavelli et al.¹¹ demonstrated that different suturing configurations produced significant differences in marginal flap stability following coronally advanced flap procedures, supporting the broader concept that suture design can influence flap biomechanics. Consistent with this principle, the greater immediate coronal advancement observed in the present study suggests that differences in anchorage configuration may also affect the extent of flap displacement achieved during coronal repositioning, an effect that may be particularly relevant in tunnel procedures, where coronal advancement is achieved while preserving papillary integrity and avoiding vertical releasing incisions.

The present study has several methodological strengths. Unlike previous clinical descriptions in which suturing techniques were evaluated as part of broader surgical procedures, the present experimental design isolated the immediate effect of suture configuration on coronal flap advancement under controlled *ex vivo* conditions. The paired design allowed direct comparison of the two techniques within the same experimental site, thereby reducing the influence of inter-site

anatomical variability. The order of suture application was randomized, and additional analyses were performed to assess potential period and sequence effects associated with sequential testing. Furthermore, a mandible-level sensitivity analysis was conducted to examine the robustness of the primary finding to possible dependence between the two sites obtained from the same mandible. Baseline flap mechanics were standardized using a universal testing machine, with all sites calibrated to the same predefined 0.60 N reference force before applying the test sutures. Identical suture material and standardized flap preparation further reduced procedural variability. Finally, coronal flap advancement was quantified directly using a digital caliper with a resolution of 0.01 mm, and all measurements were performed by the same experienced periodontist.

Several limitations of the present study should be acknowledged. First, the sheep cadaver model does not fully reproduce the anatomical and biological conditions of clinical root coverage procedures. Differences between sheep and human soft tissue characteristics may affect flap behavior, and the present experimental setting could not reproduce biological processes such as vascular perfusion, postoperative edema or shrinkage, wound healing, tissue remodeling, graft integration, and patient-related factors. Second, the study was exploratory and included only six mandibles. Because no reliable prior comparative data were available to support an effect size-based sample-size calculation, no formal power calculation was performed. The relatively small number of mandibles limits the precision and generalizability of the findings. Although the mandible-level sensitivity analysis yielded a result consistent with the primary site-level analysis, the primary analysis did not explicitly model dependence between sites from the same mandible. The mandible-level analysis served as a sensitivity check for site clustering but was not equivalent to a formal multivariable linear mixed-effects model.

Third, both suturing techniques were tested sequentially at the same anatomical site. Although application order was randomized and the flap was returned to its marked baseline position between procedures, the initial 0.60-N loading used for tension standardization, together with manipulation during the first suturing procedure, may have altered tissue behavior through viscoelastic stress relaxation and hysteresis, plastic deformation, or local microtrauma. Returning the flap to the same position does not establish that its original elastic modulus or force-displacement response was restored before the second procedure, as these mechanical properties were not reassessed. The nonsignificant period effect ($P=0.719$) and sequence effect ($P=0.480$) therefore cannot exclude carryover, particularly given the small sample.

Fourth, the visibly different suture configurations prevented blinding of the operator and outcome assessor, creating a potential risk of measurement bias. Although each measurement was immediately repeated by the same experienced periodontist, no formal intra-examiner reliability testing, such as an intraclass correlation coefficient (ICC), or measurement-error analysis was performed; therefore, measurement reproducibility could not be quantified. Finally, the study assessed only immediate coronal flap advancement and did not directly evaluate flap stability, micromotion, resistance to displacement, biological healing, or clinical root coverage outcomes. Accordingly, the findings should be interpreted as preliminary biomechanical evidence obtained under controlled ex vivo conditions.

Future studies should further characterize the biomechanical behavior of different suturing configurations using experimental models that directly quantify flap stability, micromotion, resistance to displacement, and force distribution. Studies with larger numbers of independent experimental units would also help confirm the reproducibility and precision of the present findings. In addition, clinical investigations are needed to determine whether the greater immediate coronal advancement observed with the butterfly suture is maintained after surgery and whether it

translates into meaningful differences in root coverage, gingival margin position, wound stability, or other patient-relevant clinical outcomes over time.

Conclusion

Within the limitations of this ex vivo study, the butterfly suture achieved significantly greater immediate coronal flap advancement than the sling suture in a sheep cadaver tunneling model. The observed difference was associated with a large standardized effect size, suggesting that suture configuration can influence the extent of coronal flap displacement under controlled experimental conditions. However, the clinical significance of this difference remains uncertain. These findings provide preliminary experimental evidence supporting further biomechanical and clinical studies of the butterfly suture to assess flap stability, postoperative tissue behavior, and root coverage outcomes.

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Declaration of Generative AI and AI-Assisted Technologies

During the preparation of this manuscript, the authors used ChatGPT (Sol 5.6, OpenAI) to improve the clarity, grammar, and readability of the text and to assist with the design and preparation of the graphical abstract. The authors critically reviewed and verified all AI-assisted text and graphical content and take full responsibility for the accuracy, integrity, and final content of the manuscript, including the graphical abstract.

Authors' Contributions

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Competing Interests

The authors declare no conflicts of interest.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1404.033).

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Table 1. Descriptive statistics of coronal flap advancement achieved by the two suturing techniques

Technique	Mean ± SD (mm)	Median (IQR) (mm)	Minimum–Maximum (mm)
Sling	2.85±0.69	2.84 (1.01)	1.90–4.19
Butterfly	3.48±0.64	3.51 (0.86)	2.42–4.79

SD = standard deviation; IQR = interquartile range

Table 2. Comparison of coronal flap advancement between Butterfly and Sling sutures

Comparison	Mean difference (mm)	95% CI	T (df)	P-value	Hedges' g (95% CI)
Butterfly – Sling	0.63	0.41–0.85	6.43 (11)	<0.001	1.79 (0.86–2.70)

CI: confidence interval

Hedges' g was calculated for the paired comparison using the standard deviation of the paired differences as the standardizer, with a small-sample correction applied. Positive mean differences indicate greater coronal advancement with the Butterfly suture.

Table 3. Additional analyses assessing period and sequence effects and mandible-level sensitivity

Analysis	Estimate (mm)	95% CI	t (df)	P-value
Period effect: second–first measurement	−0.08	−0.55 to 0.39	−0.37 (11)	0.719
Sequence effect: butterfly–first–sling–first treatment effect	0.16	−0.37 to 0.68	0.76 (5)	0.480
Mandible-level sensitivity analysis: butterfly–sling	0.63	0.38 to 0.88	6.52 (5)	0.001

CI: confidence interval

The period effect represents the difference between advancement measured during the second and first suturing procedures across the 12 experimental sites. The sequence effect represents the within-mandible difference in the Butterfly–Sling treatment effect between Butterfly–first and Sling–first sequences. For the mandible-level sensitivity analysis, Butterfly–Sling differences from the two sites belonging to each mandible were averaged, yielding one treatment-effect estimate per mandible.

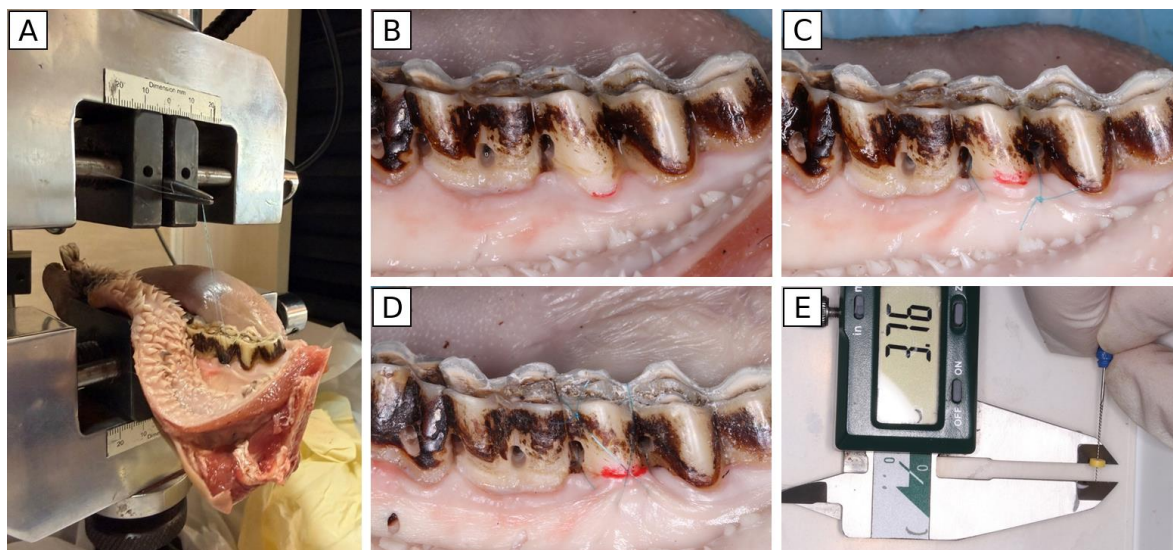


Figure 1. Experimental setup and outcome assessment. (A) Sheep cadaver mandible mounted on a universal testing machine to standardize flap tension before suturing. (B) Marking the initial gingival margin position before suture placement. (C) Butterfly suture configuration. (D) Sling suture configuration. (E) Measurement of coronal flap advancement as the distance between the initial and final gingival margin positions using a digital caliper.