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# Effect of Natural Extracts as Mouthwash on Tensile Strength of Sutures

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## ABSTRACT

**Aim:** This study focused on the effect of natural herbal mouthwashes which are dipotassium glycyrrhizinate, propolis extract and a mixture of the two, on the tensile strength of different sutures in oral surgery.

**Materials and Methods:** Two commonly used resorbable sutures polyglactin (PGA) and poliglecaprone 25 (PGCL) were evaluated in this study in two different sizes: 3/0 and 4/0. The tensile stress of the sutures was tested pre-immersion (baseline) and post-immersion at 24 h, 3 days, 1 week, and 2 weeks in different media: artificial saliva, chlorhexidine, and the natural herbal mouthwashes. Tensile strength was measured with a load set at 25 N at a standardized speed of 2 mm/min. Results were assessed using one-way and three-way analysis of variance (ANOVA) along with the Tukey post hoc test at a $p \leq 0.05$ significance level. The mean tensile strength of absorbable sutures was statistically different in the overall model for the tested experimental conditions and times ($p<0.05$). The least tensile strength was in artificial saliva where the test mouthwashes exhibited the highest tensile strength in different timeframes and suture types.

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## Introduction

The suturing of surgical wounds has a vital role in preserving tissue integrity; by ensuring that continuous closure of flap incisions is achieved. It facilitates tissue healing in an expected manner, resulting in satisfactory therapeutic results. Failure to close wounds can result in tissue tearing, esthetic concerns and overall sub-optimal healing [1,2].

Depending on the material, structure and degeneration; sutures may be classified in various ways such as, natural or synthetic, Monofilament or Multifilament and absorbable or nonabsorbable [3]. In the oral cavity, saliva, oral fluids and serum affect suture dissolution in different ways [4]. Factors including tissue quality, increased vascularity, speech and masticatory activities challenge the healing process of oral surgical sites when compared to other parts of the body [5]. In addition, due to the mouth's exposure to different types of food and liquids, the oral mucosa is likely to experience considerable pH and temperature changes [4]. Oral hygiene products also contribute to the variation of this environment.

Such products include mouthwashes which are commonly used as a chemotherapeutic measure in maintaining good oral hygiene. They can decrease plaque accumulation in a safe and effective way. They also contribute to reducing bacteria and microorganism, which in return decrease the susceptibility of dental infections. However, most mouthwashes that are currently available contain ingredients, such as triclosan, alcohol, chlorhexidine gluconate that are responsible for multiple side effects, varying from teeth discoloration to allergic reactions. To avoid such adverse reactions, the introduction of natural herbal mouthwashes has been proposed [6,7].

Various studies have aimed to study the benefits of herbal and natural mouthwashes [8]. Among these natural ingredients, dipotassium glycyrrhizinate and propolis extract have gained some attention due to their positive clinical outcomes [9]. However, their effect on the mechanical properties of suture materials used in dental surgery has not yet been investigated.

The tensile strength of sutures is one of the most important characteristics that determines the lifetime of a suture material in the oral environment [10]. In order to maintain the security of wound closer; sustaining an acceptable tensile strength of used sutures is essential [5]. Therefore, misunderstanding the mechanical properties of commonly used sutures and the effect of newly developed mouthwashes on them, can be detrimental to overall tissue healing [4].

Hence, our current study aim was to investigate the effect of dipotassium glycyrrhizinate, propolis extract and a mixture of

the two on the tensile strength and mechanical load of commonly used sutures used in dental surgeries during a two-week period.

## Materials and Methods

### Materials

Two commonly used resorbable sutures were evaluated in this study in two different sizes: 3/0 and 4/0. These types were polyglactin (PGA) and poliglecaprone 25 (PGCL) (Table 1).

**Table 1: Suture Materials Used in the Experiment**

| Suture Material | Composition | Degradation | Manufacturer |
|---|---|---|---|
| Polyglactin 910 (PGA) | Synthetic - multifilament | Resorbable | Petcryl™, Ethicon, Somerville, NJ, USA |
| Poliglecaprone 25 (PGCL) | Synthetic – monofilament | resorbable | Pectryl™, Medipac, Kilkis, Greece |

### Sample Preparation

Four hundred and twenty sutures were exposed to five different media, and the tensile strength of each was measured at four timeframes: pre-immersion, 24 h, 72 hours, 1 week, and 2 weeks post-immersion. The five experimental media were artificial saliva, chlorhexidine gluconate, 2% dipotassium glycyrrhizinate, 2% propolis extract, and a 1:1 mixture of the two. All solutions were kept at specific pH levels throughout the study.

The sutures were taken from sterile and unexpired packs, then they were measured to a length of 10 cm to accommodate the material in the testing machine. First, each suture material (n=20) was tested as dry for tensile strength before being immersed in any media. The sutures were then immersed in each media for a period of 24 h,72 hours, 1 week, and 2 weeks. Five strands of suture from each suture material were distributed according to the different time periods and media studied.

Each suture type was used to create samples that were tied using a surgeon's knot (2:1:1) followed by three regular knots around a rubber rod with a set diameter of 8mm (figure 1).

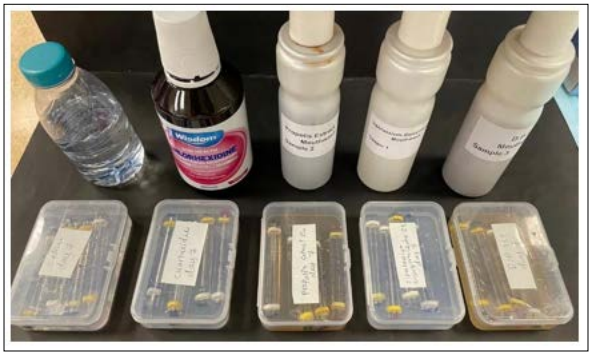

**Figure 1:** The sutures tied for each mouthwash

**Figure 2:** The Petcryl, Polyglactin 4-0 in the testing machine

On the day of testing, the sutures to be measured were removed from the rubber rod, while the rest of the specimens were kept in their containers untouched to get tested on the upcoming testing days. An Instron Universal Testing machine (UTM) was used (Instron-5965) with a load cell capacity of 50 N. Each sample was positioned vertically within the lower and upper grip fixture of a universal testing apparatus (figure2).

The testing speed was set at 25 mm/min to avoid causing damage to the sutures. The maximum load was then recorded in Newton (MaxloadN) after stretching each strand to the failure point.

To ease the discussion of results, each suture was given a group number as shown in figure3

| Group | Type of Suture |
|---|---|
| A | Petcryl, Polyglactin 4-0 |
| B | Petcryl, Polyglactin 3-0 |
| C | Petcryl Mono 3-0 |
| D | Petcryl Mono 4-0 |

**Figure 3:** Groups according to Suture Type in the Study

### Statistical Analysis

Data was analyzed using SPSS 26.0 statistical software (IBM, Chicago, IL, USA). Descriptive statistics (mean and standard deviation) was used to describe the quantitative variables (MaxLoadN and tensile strength). One-way, two-way and three-way analysis of variance (ANOVA) followed by Dunnet T3 pairwise multiple comparison tests was used to compare the mean values of MaxLoadN and tensile strength across the different time points (24 h, 72hours, 1 week, and 2 weeks), five types of media, and four types of sutures. A p-value of $\leq 0.05$ was deemed to be of statistical significance.

### Ethical Consideration

Ethical approval was obtained from College of Dentistry Research Center (CDRC)(IR0463).

## Results

The normality test was satisfied using Kolmogorov-Smirnov and Shapiro-Wilk (Shapiro-Wilk) tests which revealed p value $> 0.05$, and equality of variance which was not satisfied, (Levantine test $p<0.05$).

**Tensile Strength**

The mean tensile strength of absorbable sutures was statistically different in the overall model for the tested experimental conditions and times ($p<0.05$).

Three-way ANOVA showed that there was no significant interaction among the groups, media and time ($p= 0.184$).

Within each group, we compared the media in each time, and times within each media using 1-way ANOVA followed by Dunnett T3 pairwise multiple comparison test (MCT). However, the interaction between group, media showed significance ($p<0.01$).

**Table 2: Between-Subjects Effect Regarding Tensile Strength**

| Variable | Mean Square | F | Sig. |
|---|---|---|---|
| Group | 4658509.711 | 562.706 | .000 |
| Media | 42158.821 | 5.092 | .001 |
| Time | 20736.461 | 2.505 | .059 |
| Group * Media | 14767.952 | 1.784 | .050 |
| Group * Time | 15323.817 | 1.851 | .058 |
| Media * Time | 11227.923 | 1.356 | .185 |
| Group * Media * Time | 10124.442 | 1.223 | .184 |

**Group A**

Day 1 was not significantly different, whereas in Day 3 significant difference was observed (table 3).

Dunnett T3 pairwise multiple comparison test (MCT) showed artificial saliva with the lowest strength, where in 0.2% chlorohexidine and 1:1 mixture TS was slightly higher. However, the difference was not significant. Dipotassium Glycyrrhizinate 2% and propolis extract 2% on the other hand were higher significantly than artificial saliva (table 3).

**Group B**

No significant differences were seen in day 1 and day 14, however day 3 and 7 were significantly different among the solutions. The artificial saliva was significantly the lowest in strength compared to other solutions.

In day 7; artificial saliva and 0.2% chlorohexidine were significantly low. Propolis extract 2% and 1:1 mixture was slightly higher with no significant difference ($p=0.2$).

Dipotassium Glycyrrhizinate 2% was significantly higher than artificial saliva and 0.2% chlorohexidine ($p=0.00$).

**Groups C and D**

There was no significant difference between each time and media (Table 2).

**Table 3: Descriptive Statistics (Mean and Standard Deviation) and Dunnett T3 Pairwise Multiple Comparison Test (MCT) across the Four Time Points in Each of the Five Media Types for Each of the 4 Types of Sutures**

| Strength | | Time | | | | |
|---|---|---|---|---|---|---|
| | | Day1 | Day3 | Day7 | Day14 | |
| Group | Media | mean±SD | mean±SD | mean±SD | mean±SD | Wulsh P-value |
| A | Dipotassium Glycyrrhizinate 2% | 263.41+19 | 281.5+24.4 | 256.22+31.3 | 261.58+16.1 | 0.503 |
| | Propolis Extract 2% | 250.57+32 | 293.03+18.5 | 263.31+24.3 | 239.73+39.4 | 0.059 |
| | 1:1 Mixture | 247.32+14.4 | 251.2+48.8 | 259.64+13.9 | 238.78+31.1 | 0.506 |
| | Artificial Saliva | 251.25+14.4 | 211.33+26.7 | 223.17+6.8 | 220.07+10.6 | 0.023 |
| | 0.2% Chlorohexidine | 258.69+15.5 | 256.49+19.1 | 230.7+34.1 | 240.04+12.4 | 0.198 |
| | P-value Within Time | 0.713 | 0.005 | 0.052 | 0.173 | |
| B | Dipotassium Glycyrrhizinate 2% | 733.71+66.1 | 787.42+41.6 | 813.77+50.7 | 729.44+156 | 0.279 |
| | Propolis Extract 2% | 749.19+20.2 | 820.77+27.7 | 718.94+81.5 | 838.97+83 | 0.279 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 1:1 Mixture | 719.91+77.4 | 794.34+64.7 | 724.24+67.6 | 796.2+71.4 | 0.279 |
| | Artificial Saliva | 706.06+41.8 | 625.35+95.9 | 673.87+32.2 | 680.62+151.4 | 0.279 |
| | 0.2% Chlorohexidine | 661.64+80.4 | 816.96+104.8 | 675.91+20.9 | 755.27+75.4 | 0.279 |
| | P-value Within Time | 0.248 | 0.034 | 0.005 | 0.262 | |
| C | Dipotassium Glycyrrhizinate 2% | 604.59+182 | 635.33+68.4 | 586.87+100.3 | 523.75+45.5 | 0.104 |
| | Propolis Extract 2% | 715.71+84.4 | 662.52+63.7 | 726.98+79.2 | 545.83+97.9 | 0.104 |
| | 1:1 Mixture | 632.24+108.2 | 716.84+55.8 | 497.79+198.8 | 450.49+193.2 | 0.104 |
| | Artificial Saliva | 600.7+80.3 | 545.53+95.3 | 552.44+145.8 | 553.69+32.5 | 0.104 |
| | 0.2% Chlorohexidine | 537.18+391.5 | 480.89+251.5 | 574.46+127.2 | 573.05+96.8 | 0.104 |
| | P-value Within Time | 0.376 | 0.061 | 0.144 | 0.457 | |
| D | Dipotassium Glycyrrhizinate 2% | 254.89+48.5 | 258.1+42.6 | 206.1+50.4 | 269.43+73.9 | 0.360 |
| | Propolis Extract 2% | 246.68+92.3 | 295.02+46.1 | 248.22+66.2 | 238.88+26.9 | 0.360 |
| | 1:1 Mixture | 288.74+55.7 | 322.13+35.1 | 223.34+67.6 | 215.34+62.9 | 0.360 |
| | Artificial Saliva | 243.41+31.1 | 260.5+70.9 | 250.76+71.6 | 296.66+35.5 | 0.360 |
| | 0.2% Chlorohexidine | 298.29+81.6 | 224.35+121.2 | 245.08+50.1 | 265.96+40.8 | 0.360 |
| | P-value Within Time | 0.574 | 0.265 | 0.744 | 0.158 | |

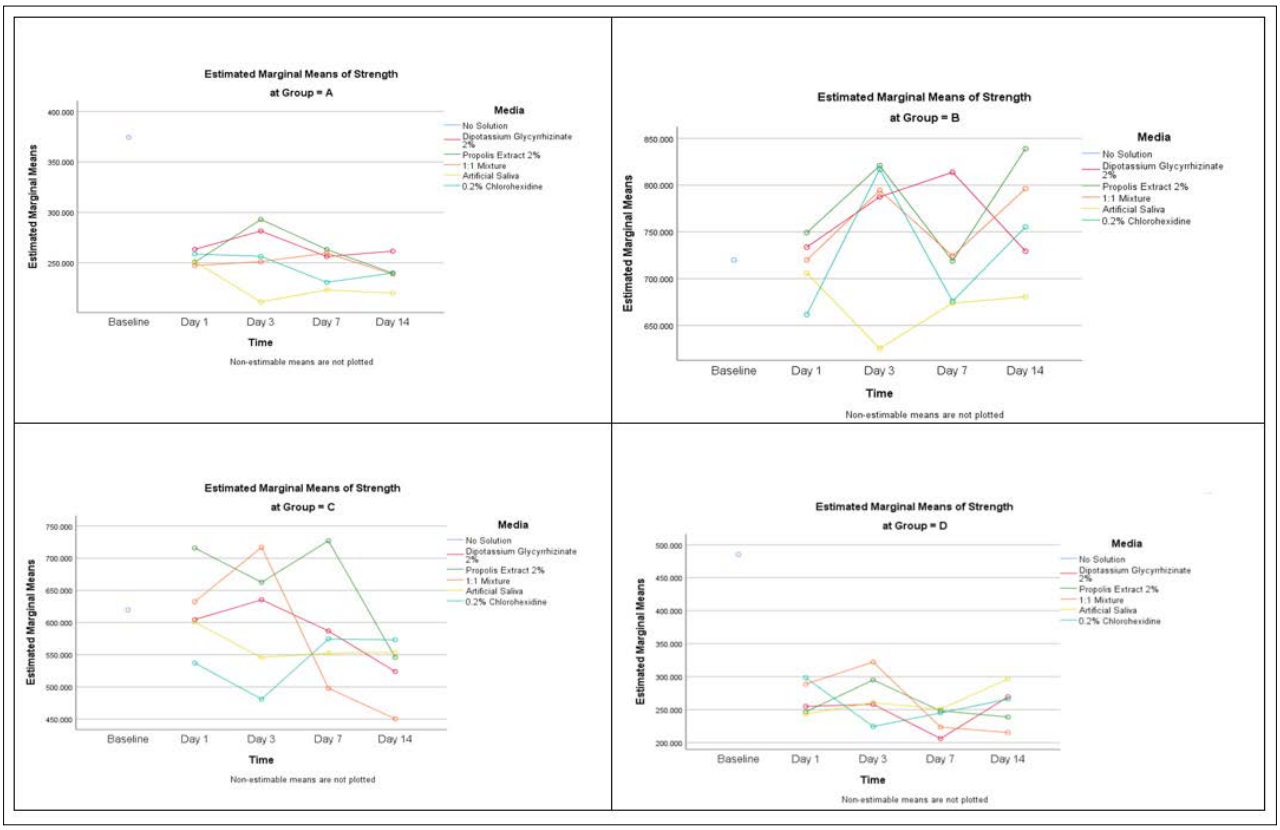


**Figure 4:** Tensile Strength of Studied Sutures Immersed in Five Media for the Different Time Periods

Point of Breakage (Figure 5)

In group A, the greatest percentage of breakage was on the knot except for:

Dipotassium Glycyrrhizinate 2% in which all 100% of breakage was on the knot except on day 1 and 7 it was far from the knot by 40% ,20% respectively.

Propolis extract 2% had 100% of the breakage on the knot, except for day 3, which showcased 20% of slippage of the sutures, whereas Artificial saliva at day 1 was 20% far from the knot.

In group B, the majority percentage of breakage was on the knot by 100% except for:

Dipotassium Glycyrrhizinate 2% on day 1 which showcased breakage far from the knot by 20%. Propolis Extract 2% on day 3 and 14 had slippage by 20% and 40% respectively, while 1:1 mixture on day 14 had slippage by 40%. In Artificial Saliva on day 14, most of the breakage was on the knot by 80% and near the knot by 20%. Whereas 0.2% chlorohexidine on day 1 had breakage by 20% near the knot and 20% far from the knot.

In group C, the majority of breakage was also on the knot, except for: Dipotassium Glycyrrhizinate 2% in Day 1 in which showcased breakage near the knot and far from the knot by 20%, 40% (respectively).

Day 3 and Day 14 had slippage of the knot occurred by 80%, 20% (respectively), while Day 7 had 60% of breakage occur far from the knot.

In addition, Propolis extract 2% had 20% of breakage far from the knot in Day 1.

In Day 3, slippage of the knots and breakage far from the knot occurred by 60%, 20% (respectively). In Day 7, 40% of the breakage was far from the knot, while in Day 14; 20% had slippage of the knot.

The 1:1 mixture at day 1, 3, 7, 14 had breakage occur far from the knot by 20%, 40%, 20% and 40%(respectively). At Day 7 and 14 both of which showcased 20% of breakage near the knot. Artificial saliva at Day 7 had 20% of slippage, 40% were far from the knot, and Day 14 had 20% of slippage, whereas 60% were far from the knot.

0.2% chlorohexidine at Day 1 had breakage near the knot by 40%. Day 3 was 40% on the knot where slippage and breakage near and far from the knot were each 20%

For group D, Dipotassium Glycyrrhizinate 2% showcased 20% of breakage far from the knot in Day 1 and 7 while Day 3 had 20% slippage of the sutures.

Propolis extract 2% at Day 1 had 20% of occurrence for breakage far from the knot, Day 3 with 20% slippage of the sutures and Day 7 with 40% of the breakage near the knot.

For the 1:1 mixture; the breakage point was 20% far from the knot at Day 1, and breakage near the knot at Day 7 also showcased 20% of occurrence.

Artificial saliva had a greater percentage of breakage far from the knot at Day 1,3,7 by 20%,20%,40% (respectively), whereas at Day 14; had 100% of the breakage occurring on the knot.

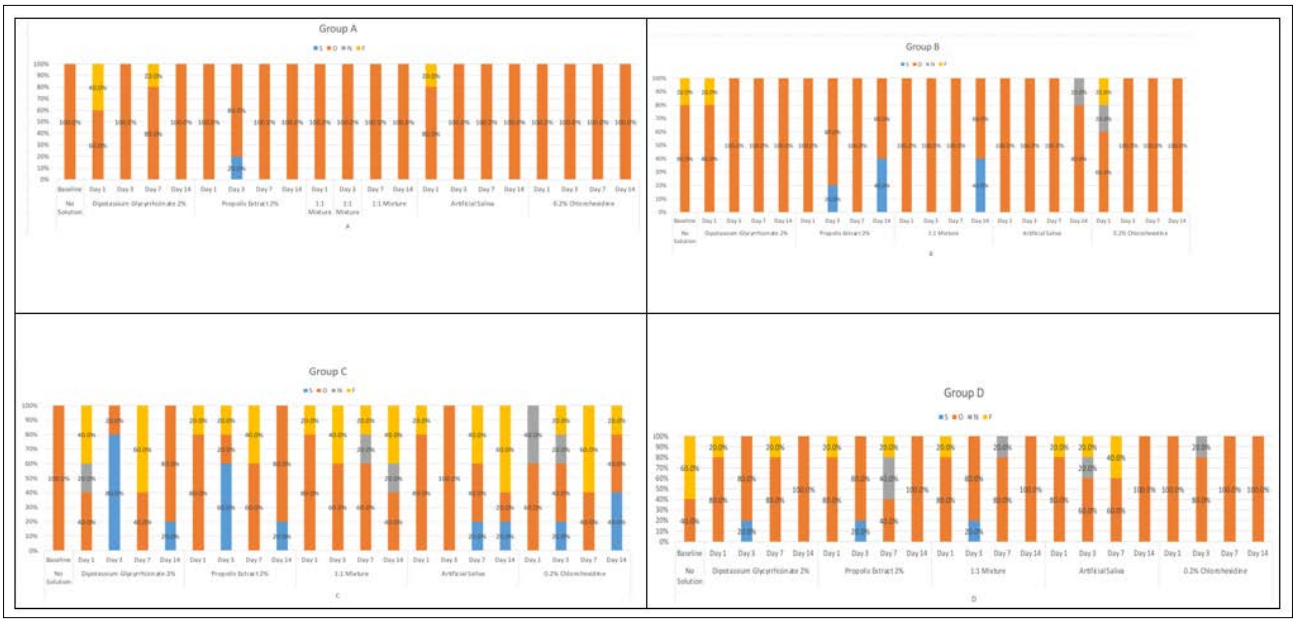


**Figure 5:** Point of Breakage within the Different Medias in the Different Time Zones

## Discussion

This study aimed to compare the effect of naturally extracted mouthwashes on commonly used sutures in oral cavity. Dipotassium Glycyrrhizinate (DPG) and propolis extracts were shown to have anti-inflammatory effects [11]. Their effects on sutures integrity were compared to commonly used mouthwash in this study.

While both artificial saliva and chlorhexidine can influence suture strength, studies suggest artificial saliva has a more detrimental effect [4]. Artificial saliva seems to consistently weaken sutures across various materials. In a study by R.Ferguson et al , it

was observed that a significant reduction in tensile strength for Vicryl and chromic gut suture types immersed in artificial saliva compared to dry controls[12]. Furthermore, despite the suture type differences, AlSarhan et al. concurred the effect of artificial saliva in his research, showing a significant increase in tensile strength when immersed in chlorhexidine and Listerine solutions compared to artificial saliva and the dry condition for Vicryl 4-0 and 5-0 [1]. In addition, Alshehri at al. has interestingly reported more rapid tensile strength loss after being exposed to saliva, especially after 7 days with a 5-0 PLG (VicrylTM, Ethicon) suture [13]. Similar to our study results; the suture groups used that were immersed in artificial saliva in our experiment showcased the lowest tensile strength compared to chlorohexidine. On the other hand, some research indicates no significant difference in tensile strength comparing chlorhexidine to saliva [1]. Abullais et al. who also tested other types of solutions; one of which included chlorhexidine; found that chlorhexidine was statistically nonsignificant with Polytetrafluoroethylene (PTFE), Vicryl, and Prolene sutures regarding their tensile strength [4].

In our experiment, the results showcased significant lower tensile strength for sutures soaked in artificial saliva across all days measured, compared to other mediums; while Dipotassium Glycyrrhizinate 2% (DPG) and propolis extract 2% revealed significantly higher tensile strength. Inflammation can break down collagen, a crucial component for suture strength. Therefore, by mitigating inflammation around the suture site, DPG could potentially help maintain the integrity of the suture material [11]. Similarly, propolis extract, a natural product with antibacterial and wound healing effects, has been shown to improve suture strength in animal studies [14]. Studies also suggest it can stimulate the growth of fibroblasts; the cells responsible for collagen production [15]. This enhanced collagen deposition could reinforce the suture and surrounding tissue, leading to improved tensile strength. While the exact mechanisms require further investigation, both DPG and propolis extract offer exciting possibilities for developing postoperative mouthwashes that promote healing and optimize suture performance.

This study also investigated the impact of suture material and gauge on tensile strength. They compared two types of sutures: monofilaments (Poliglecaprone 3-0 and Petcryl Mono 4-0) and poly-filaments (Vicryl and polyglactin, divided by gauge 3-0 and 4-0). Interestingly, there was no significant difference in tensile strength between the monofilament gauges (3-0 vs. 4-0) across all time points and immersion mediums.

However, the poly-filaments exhibited a more complex pattern. Their tensile strength peaked on day 3 when immersed in Dipotassium Glycyrrhizinate 2% and propolis extract 2%. Conversely, artificial saliva seemed to reduce their tensile strength. This finding contrasts with another study by S. Khiste, where Vicryl sutures showed a temporary higher tensile strength with the finer gauge (4-0) at day 10, but later the difference became negligible by day 14 [16].

Focusing on the factor of time, the observed decline in tensile strength of sutures exposed to artificial saliva compared to later days remains a topic of investigation. While the specific reasons need further exploration, a few possibilities can be explored based on existing research. Firstly, artificial saliva compositions often contain enzymes that mimic salivary amylase and lysozyme, known to break down carbohydrates and proteins respectively [17]. These enzymes might accelerate the initial degradation of suture materials, particularly those with susceptible components. Secondly, the constant presence of these enzymes in artificial saliva could lead to a more sustained degradative effect compared to natural saliva, where enzymatic activity fluctuates with eating and drinking [18]. S. Khiste et al. observed a similar pattern, where sutures initially lost strength faster in artificial saliva but displayed a more gradual degradation later [16]. This suggests that the initial burst of enzymatic activity in artificial saliva might be responsible for the steeper decline in tensile strength at day 7. Additionally, the specific formulation of artificial saliva used in the study could play a role. Different artificial saliva recipes vary in their enzyme concentrations and other components, potentially affecting their interaction with sutures. Synthetic sutures (e.g., nylon, polyester) are generally less susceptible to degradation by enzymes or chemicals present in artificial saliva compared to natural sutures (e.g., silk, collagen) [19].

The knot configuration of sutures is another important factor that influences suture stability (20). The present study used the surgeon's knot in all samples to reduce knot untying [21]. While the surgeon's knot is a reliable choice for its security and stability, it might introduce a confounding factor in studies examining the effect of mouthwash on suture tensile strength. This knot type, although strong, can potentially mask subtle differences in how mouthwashes interact with the suture material itself. For instance, a mouthwash might slightly reduce the grip between suture fibers, but a secure knot like the surgeon's knot could compensate for this by maintaining overall stability during the tensile strength test [22]

Our current experiments included several findings. In polyglactin 4-0 group, there was a greater tendency for suture breakage on the knot. On the other hand, polyglactin 3-0 group, although showed a great percentage of breakage on the knot, it also had a multi-configuration of breakage, and a higher percentage of far breaking point and the occurrence of slippage than the previous group which occurred mostly in propolis extract. This could be attributed to the viscosity of the propolis mouthwash.

Highly viscous mouthwashes might create a lubricating film around the suture, reducing friction and potentially affecting its grip. This could lead to a slight decrease in tensile strength [23]. However, more research is needed to confirm this effect.

In mono filament groups of 3-0 and 4-0, untying of knots occurred more frequently that in the poly-filament suture groups. Many researchers highlight the drawbacks of monofilament absorbable surgical threads, including their lower strength compared to poly-filament threads and a significant decrease in strength when knotted. For example, while polypropylene may lose 8-15% strength in a knot, monofilaments can experience a 40-50% loss. To address the low reliability of knots with monofilaments, it is recommended to use at least six knots when tying them [24].

This phenomenon explains the ability of multifilament braided sutures such as Vicryl to resist knot untying compared to monofilament sutures like Monocryl under increased tensile loads. This result is demonstrated in Kim et al. who reported that monofilament sutures showed a tendency to untie easily after being loaded to failure [24]. Pcheliakov et al. also found that Vicryl has the highest force in the knot, due to which it better holds the wound edges in comparison with other sutures. However, alongside the risk of infection and deep penetration of the microflora in the tissue, this material is also proven to have high wicking. High knot force with low elongation might indicate the formation of microcracks on the surface of the material because of the load, and that leads to more bacterial absorption. Prolene and Glycolon, as

monofilament threads, had the lowest capillarity, and they are less prone to infection of surrounding tissues. With a high extensibility, followed by a weakening of the tension of the wound edges, these materials continue to have a high susceptibility to the formation of bacterial plaque [25].

The current experiment had some limitations regarding the inability to perfectly replicate the conditions of the oral cavity, such as the interaction of these mouthwashes with the serum fluids and saliva. In addition, patients' conditions i.e., medical illnesses and oral habits; influence the retention of suture materials. However, our study revealed a noteworthy difference in how the mouthwashes impacted suture tensile strength. While artificial saliva reduced tensile strength, Dipotassium Glycyrrhizinate and propolis extracts did not exhibit this adverse effect. Given this observation, further investigation in a clinical setting (in vivo) would be valuable to confirm these findings in a more realistic environment.

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