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ORIGINAL PAPER

An in vitro study for the evaluation of morphological and biochemical characteristics of absorbable sutures coated with genistein and nicotine

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ABSTRACT

Introduction and aim. Inflammation, cell proliferation, matrix deposition, and tissue remodeling are all elements of the well-structured and well-coordinated process of wound repair. The aim of this study was to analyze the effect of genistein and nicotine on polyglycolic acid (PGA) and vicryl sutures.

Material and methods. Genistein and nicotine were isolated and solution was prepared and the suture material PGA and vicryl were immersed in the solution and dried. They were tested for their tensile strength and degradation values after immersion in artificial saliva (on the first day and on the 14th day). The sutures were also seen under a scanning electron microscope (SEM) for its uniform coating and the mixture formulation of genistein and nicotine were tested for their anti-inflammatory and antioxidant activity using protein denaturation assay and 2,2-diphenyl-1-picrylhydrazyl assay respectively.

Results. Nicotine has a high anti-inflammatory activity on the suture material, whereas Genistein has an insignificant anti-inflammatory effect. The mixture formulation has a relatively similar anti-inflammatory effect when compared to the control. The SEM analysis shows a uniform coating of the formulation on the PGA and vicryl sutures. In comparison, PGA has shown lesser tensile strength and hence higher degradation ability.

Conclusion. Nicotine and Genistein affect the tensile strength and degradation properties of the sutures.

Keywords. absorbable sutures, antiinflammatory, antioxidant, genistein, nicotine, tensile strength

Introduction

Inflammation, cell proliferation, matrix deposition, and tissue remodeling are all elements of the well-structured and well-coordinated process of wound repair. Until the wound develops enough tensional strength to prevent dehiscence, sutures serve to maintain tissue proximity.[1] The success of the surgical treatment is influenced by the proper closure and stabilization of the surgical wound margins. The behavior of sutures used in oral and maxillofacial surgery depends on the quality of the tissues involved, the presence of saliva, and particular bacteria. They serve as a conduit connecting the tissues' internal and exterior regions, which affects how well wounds heal.[2] It has been noted that general characteristics of

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the patients (namely, gender and age) and of the wounds (that is, length and site) seemed to be the two principal risk factors responsible for local wound complications, rather than suture materials and different surgical techniques. A suitable suture material shouldn't obstruct connective tissue organization or cellular growth.

For the majority of oral surgical procedures, suturing a previously raised flap is used for initial wound closure. A number of suture materials are available for this purpose and can be categorized based on their origin (organic and synthetic) or on how long they will last in the host tissues (absorbable and nonabsorbable).[3] A strong suture that generates stable knots is the perfect suture. It does not stimulate infection and just mildly inflamed the tissue. Although no single suture combines all of these characteristics at once, careful suture selection aids in obtaining better outcomes. In order to avoid scarring and poor wound healing and to achieve good results, proper suturing technique is crucial.[4] The aforementioned elements can be enhanced and regulated to create a setting that is close to optimal, although research is still being done to find the "ideal" suturing material. Naturally, the best suturing material will vary depending on the clinical setting. The so-called ideal suture material has characteristics like appropriate approximation, support, and low immunogenicity, which are significant. Suture material must have the following characteristics in order to function properly: knot safety, stretch capacity, tissue reactivity, and wound safety.[5] Along with the chosen surgical and suturing methods, the choice of suture material may also have an impact on how quickly the soft tissues that were incised, recover. Three case reports of complications following the use of a subepithelial connective tissue graft, where an abscess developed after the first healing phase, were documented in Vastardis and Yukna's study.[6] According to this study's findings, the abscesses may have been brought on by a stitch abscess or a reaction to the suture material utilized for the submerged sutures. Thus, when establishing a treatment strategy for oral surgical operations, the choice of suture material should be taken into account.[6]

In tobacco leaves, nicotine, a natural substance, serves as a botanical insecticide. It is the main tobacco alkaloid, making up roughly 95% of the overall alkaloid content and appearing in commercial cigarette tobacco to the level of about 1.5% by weight.[7] While cigars and chewing tobacco only have roughly half the nicotine concentration of cigarette tobacco, oral snuff and pipe tobacco have nicotine concentrations that are comparable to cigarette tobacco.[8]

The medical research world has recently given genistein, an isoflavone derived from soy, a great deal of attention. It was discovered that this substance was a powerful agent in the prevention and treatment of cancer as well as other chronic diseases.[9] The ATP-using enzymes tyrosine-specific protein kinases, topoisomerase II, and those involved in phosphatidylinositol turnover are all inhibited by genistein at the molecular level. An estrogen receptor-mediated mechanism is one way that genistein can work.[10] Genistein affects cells at a level one step higher, or at the cellular level, where it induces apoptosis and differentiation in cancer cells, suppresses osteoclast and lymphocyte activity, reduces cell proliferation, modifies cell cycle, has antioxidant effects, and inhibits angiogenesis.[11] The key benefits of genistein as a possible medication are because of its numerous actions in living cells and its extremely low toxicity.

A synthetic, multifilament, and absorbable suturing material is called vicryl (polyglactin 910). The substance most frequently used in dentistry is this one. Due to the fact that it prevents plaque from adhering and is convenient for handling, it has been demonstrated to be clinically effective.[12] Additionally, it does not have a strong local reaction. Vicryl rapide helps human wounds heal more quickly by reducing the chances of dehiscence and causing milder local reactions.[13]

Aim

The aim of this study was to:

- prepare a genistein and nicotine extract and coat the sutures with the formulation
- to test the antioxidant and anti-inflammatory activity of the solution
- to analyze the coating of genistein and nicotine on PGA and vicryl sutures
- to test the effect of these components on the tensile strength of the suture samples at day 1.
- to test the tensile strength of the suture samples on the 14th day of immersion in artificial saliva

Material and methods

Preparation of extract

Here in this study the Genistein is taken in the form of soybean (Fig. 1). Soya powder and nicotine was bought from an organic shop. 30 g of soybeans powder was weighed using a weighing machine and added 80 mL of water and 200 mL of ethanol to it by measuring in the 100 mL measuring cylinder in a conical flask. Then it was kept in an orbital shaker (Sisco India, Mumbai, Maharashtra, India) for 24 hrs. 30 g of nicotine was also diluted with 40 mL ethanol in a conical flask and then kept in an orbital shaker for 24 hrs.

Preparation of the suture materials

The extract of genistein and nicotine prepared was taken in two different labeled test tubes. PGA (Surucry|, Gujarat, India) and vicryl sutures (Ethicon, Mumbai, India) were used for the study.

The sutures were divided for analysis into the following groups:

- group I – control PGA – uncoated
- group II – control vicryl – uncoated
- group III – vicryl (4.4 pH) – coated with genistein and nicotine
- group IV – vicryl (7.2 pH) – coated with genistein and nicotine
- group V – PGA (4.4 pH) – coated with genistein and nicotine
- group VI – PGA (7.2 pH) – coated with genistein and nicotine

The sutures (except control group) were immersed in the genistein and nicotine extract for 6 hours (Fig. 2 and 3) and placed in an orbital shaker for a uniform coating of extract over the sutures followed by air drying in a Petri dish for 3-4 hours (Fig. 4).

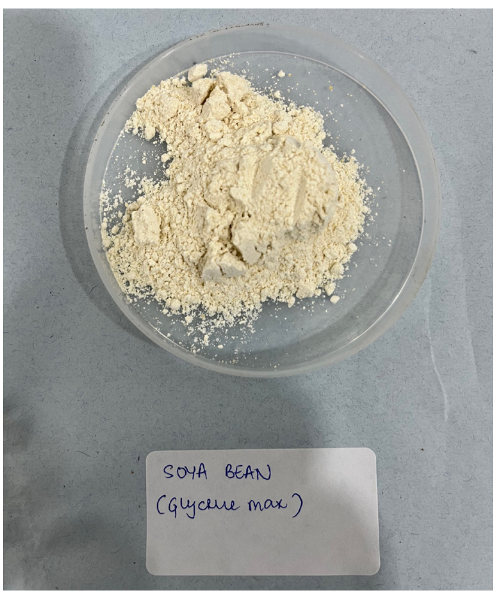

Fig. 1. Showing soybean powder used to obtain genistein extract

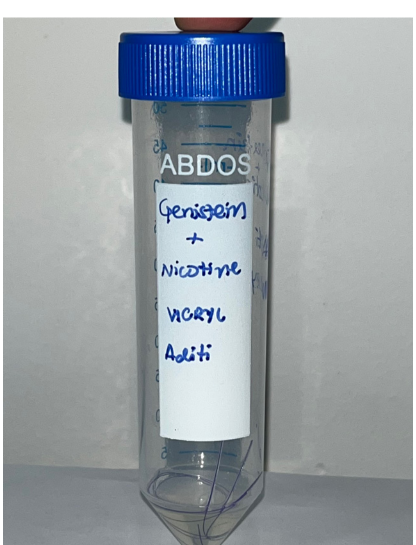

Fig. 2. Showing the Vicryl suture materials immersed in the genistein nicotine mixture

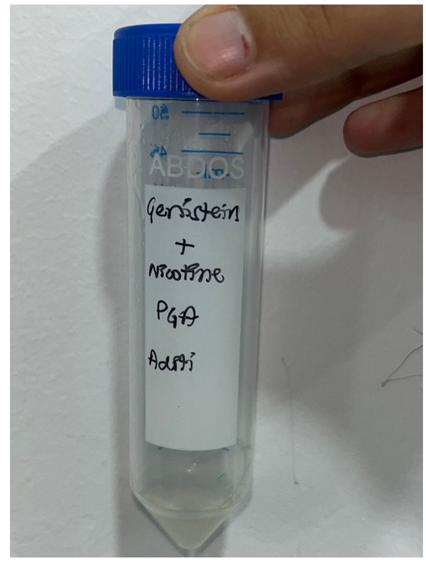

Fig. 3. Showing the PGA suture materials immersed in the genistein nicotine mixture

Fig. 4. Showing the air drying of the suture materials post immersion

Preparation of artificial saliva

Before testing the samples for tensile strength, and degradation study, the suture materials were kept in artificial saliva for 24 hours. The artificial saliva of 1000 mL was prepared by the mixture of 8.035 g of sodium chloride, 0.355 g of sodium bicarbonate. 0.225 g of potassium chloride, 0.231 g of potassium hydrogen phosphate and 0.311 g of magnesium chloride, was added to 40 mL of 1 M hydrochloric acid along with 0.292 g of calcium chloride, 0.072 g of sodium sulfate, 6.118 g of Trizma Base and 1 M hydrochloric acid. The prepared artificial saliva was divided into 2 separate beakers of 500 mL with 7.4 pH and 500 mL with pH 4.4.

Analysis

Anti-inflammatory activity of the formulation was done using protein denaturation assay – the formulation of genistein and nicotine, genistein extract, nicotine extract were screened for anti-inflammatory activity by using inhibition of protein denaturation assay. The 500 μg of control drug and test compounds (formulation of genistein and nicotine, genistein extract, nicotine extract) were dissolved in a minimum amount of dimethyl formamide (DMF) and diluted with a phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.5%. Test solution (1 mL) containing different concentrations of drug was mixed with 1 mL of 1 mM albumin solution in a phosphate buffer and incubated at 27±°C in the BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60±10°C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer, Konica Minolta CM 5, Gujarat, India). Percentage of inhibition of denaturation was calculated from control where no drug was added. The diclofenac sodium was used as a standard drug. Percent inhibition was calculated using the following formula:

$$\% \text{ of inhibition} = ((\text{control OD} - \text{sample OD}) \cdot 100)/(\text{control OD})$$

where: control OD – optical density of control; sample OD – optical density of test sample

Antioxidant activity of the formulation was done using DPPH assay – the DPPH (1,1-diphenyl-2-picryl-hydrazil) free radical scavenging activity of formulation of genistein and nicotine, genistein extract and nicotine extract was determined. 50 μg of 4 different extracts (genistein, nicotine, genistein + nicotine formulation and control) were taken in 4 test tubes and mixed with 1 mL of 0.1 mM DPPH in methanol solution and 450 μL of 50 mM Tris-HC1 buffer (pH 7.4) and incubated for 30 minutes. After incubation, the reduction in the number of DPPH free radicals was measured based on the absorbance at 517 nm. BHT was used as control. The percentage of inhibition was calculated from the following equation:

$$\% \text{ of inhibition} = ((\text{absorbance of control} - \text{absorbance of test sample}) \cdot 100) / (\text{absorbance of control})$$

Extract coated suture threads were evaluated for their morphological characterization using Scanning electron microscope (Jeol, JSM-IT800 NANO SEM, USA).

Tensile strength of the coated and uncoated (control) suture samples was assessed using INSTRON Universal Testing Machine E-3000 on day 1 of immersion in artificial saliva. All the data generated by the measurements were represented as mean and standard deviation. SPSS software version 23.0 (IBM, Armonk, NY, USA) for Windows was used for the statistical analysis. Control and coated groups were analysed with a one way ANOVA and p value <0.05 was considered to be statistically significant.

Degradation analysis was done by immersing the suture samples in artificial saliva at two different pH i.e. 7.2 and 4.4 for 14 days. This reflects 4.4 for the pediatric patients whereas 7.2 is for adult patients. Their tensile strength is tested again on day 14 of immersion to assess the degradation property of the sutures.

Results

Figure 5 represents the antioxidant effect of genistein and nicotine and the mixture formulation showed that when compared to the control ascorbic acid (90%), genistein (64%) showed a relatively higher level of antioxidant potential whereas nicotine (31%) showed very less. As a mixture (18%), nicotine will reduce the antioxidant activity of genistein as well. Figure 6 represents the anti-inflammatory effect of genistein and nicotine and the mixture formulation. Nicotine (92%) showed the highest activity when compared to the control group that is diclofenac (90%). Since genistein (63%) had a lower anti-inflammatory activity, it lowered the anti-inflammatory capacity of the genistein-nicotine mixture (81%) as well. Figures 7–10 are the SEM images revealing uniformly coated with the genistein- nicotine mixture on the suture materials. This increased the surface area of the suture, which in turn increased the healing effects of the suture on the tissues.

In Table 1, it can be seen that the Group II suture sample had the highest tensile strength (1451.71 MPa) at the maximum force of 9.3N. In comparison, the Group I suture sample had a lesser tensile strength (993.16 MPa) at a lesser maximum force of 6.38N.

The coated suture samples of vicryl and PGA at both pH values (4.4 and 7.2) have shown a lesser tensile strength when compared to the control group. This means that the coated samples will break more easily post application of force as compared to the control group.

In Table 2, it can be observed that the Group II suture sample had the lowest probability of degradation, since it had the highest tensile strength (1430.79 MPa) on the 14th day of immersion in artificial saliva. In comparison, the Group I suture sample had a lesser tensile strength (959.07 MPa) and hence degraded faster. The coated suture samples showed lesser tensile strength and therefore higher degradation values at 4.4 and 7.2 pH. While comparing the pH, it can be observed that the coated vicryl suture degraded faster at a lower pH (4.4) whereas the coated PGA suture degraded faster at a higher pH (7.2).

Pair wise comparison of PGA and vicryl groups through one way ANOVA analysis was done (Table 3). PGA sutures showed p value as 0.0363 which is statistically significant. ANOVA analysis of the Vicryl sutures showed p value as 0.0278 which is also statistically significant.

Antioxidant activity

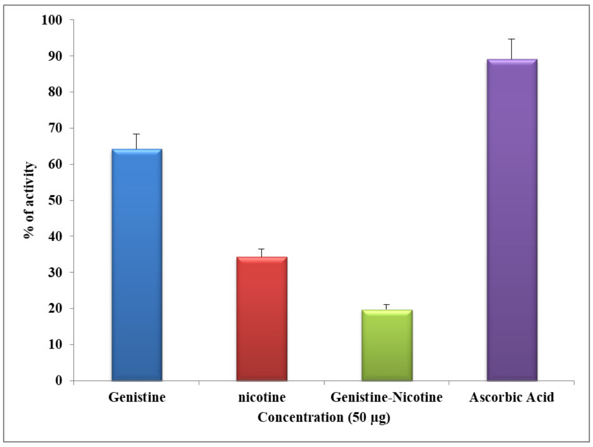

Fig. 5. Graphical representation of the antioxidant activity of the suture samples

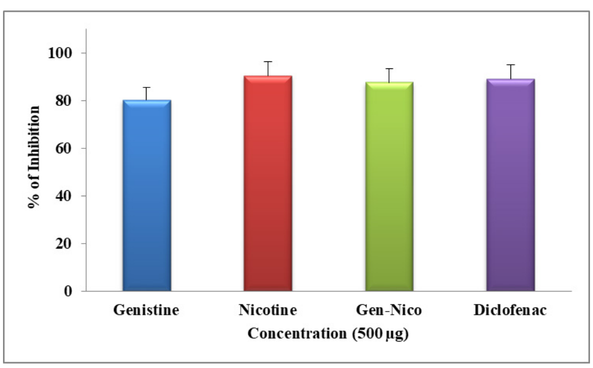

Fig. 6. Graphical representation of the anti-inflammatory activity of the suture samples

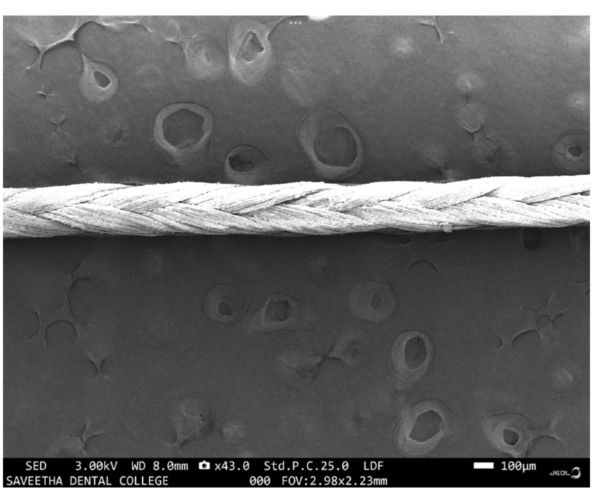

Fig. 7. Shows the SEM analysis of PGA at 37x magnification. It shows a uniform layer of the genistein and nicotine mixture on the PGA suture

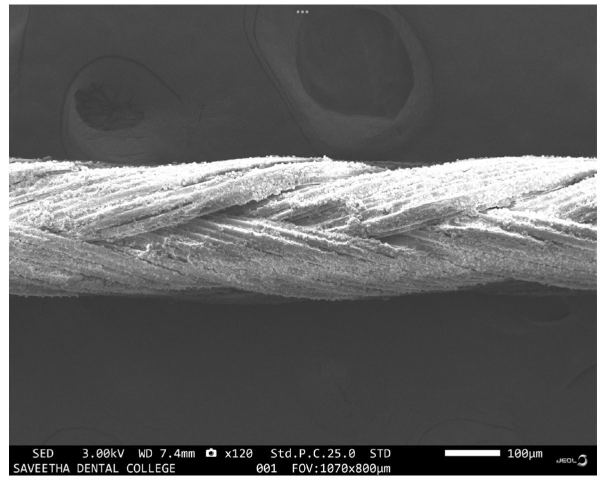

Fig. 8. Shows the SEM analysis of PGA at 120x magnification. It shows a homogenous and even coating of the genistein and nicotine formulation

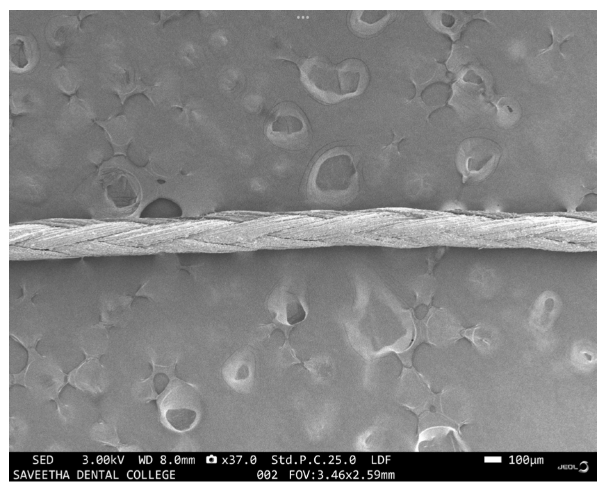

Fig. 9. Shows the SEM analysis of vicryl at 37x magnification. It shows a uniform layer of the genistein and nicotine mixture on the vicryl suture

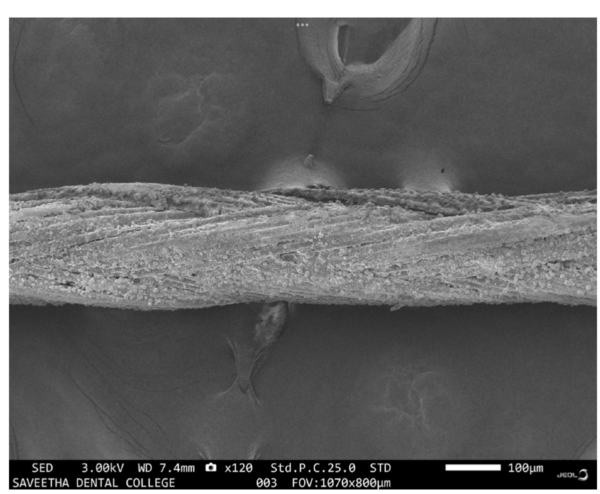

Fig. 10. Shows the SEM analysis of vicryl at 120x magnification. It shows a homogenous and even coating of the genistein and nicotine formulation

Table 1. Tabular representation of tensile strength of the coated suture samples at different pH values (4.4 and 7.2) compared to the control uncoated values. The values compared are maximum force (N), tensile stress at tensile strength (MPa) and tensile strain (displacement) at break (standard)

Sample group	Specimen label	Maximum force (N)	Tensile stress at tensile strength (MPa)	Tensile strain (displacement) at break (standard) (%)
Group I	PGA (uncoated) (control)	6.38±0.51	993.16±1.21	11.78±0.93
Group II	Vicryl (uncoated) (control)	9.30±0.83	1451.71±2.51	22.94±1.22
Group III	Vicryl (coated) (pH 4.4)	7.88±0.61	1231.48±1.73	12.68±0.87
Group IV	Vicryl (coated) (pH 7.2)	6.87±0.24	95.68±1.82	16.00±0.94
Group V	PGA (coated) (pH 4.4)	4.09±0.48	639.73±1.49	7.39±0.38
Group VI	PGA (coated) (pH 7.2)	5.26±0.75	817.92±2.31	7.60±0.42

Table 2. Tabular representation of degradation results (on the 14th day of immersion in artificial saliva) of the coated suture samples at different pH values (4.4 and 7.2) compared to the control uncoated groups

Sample groups	Specimen label	Tensile stress at break (standard) (MPa) (degradation at 14th day)
Group I	PGA (uncoated) (control)	959.07±2.45
Group II	Vicryl (uncoated) (control)	1430.79±3.27
Group III	Vicryl (coated) (pH 4.4)	1190.87±2.91
Group IV	Vicryl (coated) (pH 7.2)	1067.11±2.34
Group V	PGA (coated) (pH 4.4)	635.21±1.85
Group VI	PGA (coated) (pH 7.2)	818.98±2.27

Discussion

In the present study, it can be observed that nicotine has a high anti-inflammatory activity on the suture material, whereas genistein has an insignificant anti-inflammatory effect. The genistein-nicotine mixture formulation has a relatively similar anti-inflammatory effect when compared to the control. In accordance with the antioxidant effect, genistein has shown to have high results whereas nicotine shows the opposite. The mixture formulation shows a relatively low antioxidant effect when compared to the control. The SEM analysis shows a uniform coating of the formulation on the PGA and vicryl sutures. Tensile strength testing concludes that the vicryl control group has the tensile strength and hence the lowest degradation capacity. The coated vicryl suture samples at different pH have also shown similar results. In comparison, PGA has shown lesser tensile strength and hence higher degradation ability.

Table 3. Tabular representation of pairwise comparison and statistical significance of PGA (group I, V, VI) and vicryl (group II, III, IV) groups through one way ANOVA analysis*

Sample group	Specimen label	Tensile stress at tensile strength (MPa)
Group I	PGA (uncoated) (control)	991.0±4.839
Group II	Vicryl (uncoated) (control)	1454±17.72
Group III	Vicryl (coated) (pH 4.4)	1235±10.69
Group IV	Vicryl (coated) (pH 7.2)	93.23±4.895
Group V	PGA (coated) (pH 4.4)	639.4±7.925
Group VI	PGA (coated) (pH 7.2)	820.0±8.934
Pair wise comparison of PGA and vicryl groups through One Way ANOVA analysis		
Sample groups	Specimen label	p^a
Group I, V, VI	PGA groups	0.0363
Group II, III, IV	vicryl groups	0.0278

* ^{a} – p value<0.05, therefore statistically significant

For dental and medical surgical procedures, a variety of suture materials are available. However, it is crucial for surgeons to understand the suture material's properties, the biologic processes of healing, and how the suture material interacts with the surrounding tissues.[14] The need for a suture that will hold its strength until the tissues of the previously raised surgical flaps regain enough strength to bind the incision edges together makes this a crucial challenge.[15] Research information on the efficacies of various materials is still disputed and conflicting as of this writing. The goal of the current study was to analyze the suture responses to various food materials.

For many years, silk has been the suture material of choice for dentistry and other surgical treatments. The authors contend that silk should not be regarded as a "material of choice" for oral surgical treatments, despite the fact that it is less expensive and easier to handle than other nonabsorbable suture materials.[16] Studies on the effects of sutures on oral tissue have demonstrated significant inflammatory responses, which are more pronounced with silk and cotton and less pronounced with other materials such nylon, polyester, ePTFE, polyglecaprone 25, PGA, and vicryl.[17]

In a study conducted by Varma et al., silk and polyamide sutures were coated with hyaluronic acid and compared to the control (chlorhexidine) and tested for their tensile strength. They used the Tinius Olsen Universal Testing Machine for testing the tensile strength. As a result, polyamide showed better stability than silk and hyaluronic acid did not alter the tensile strength of either suture material pre and post immersion.[18] This is contradictory to our study, where this is a significant difference in the tensile strength of the suture material after coating. In another study the physical and functional properties of polyglactin 910 suture with and without triclosan.[19] The authors concluded that the addition of triclosan did not affect the handling properties or performance characteristics of either suture, and the breaking strength was same for both sutures, ranging from 79% on day 14 to 5% on day 35. In our study, the tensile strength decreased considerably from day 1 of immersion to day 14 of immersion, when the breaking point of the sutures were tested. A study done by Wu et al. concluded that a surface coating of polylactic acid and polycaprolactone on commercially degradable polydioxanone suture maintained 20% of its original tensile strength after 12 weeks of implantation. In addition, in vivo results of PCL-coated sutures also showed less inflammatory cell infiltration and less surface inflammation.[20] In the present study, we have similar results in which the tensile strength is reduced after immersion in the Genistein and nicotine solution. In another study, two biodegradable polymers with different degradation rates, such as polycaprolactone (PCL) and PGA, were applied to carry the drug of tea polyphenol (TP). The drug-loading finishing solution which is made of PCL/PGA carriers and TP, was coated on the PLA suture. With the increasing of PCL in drug-carriers, the strength of suture gradually increases. At 70/30 of PCL/PGA, the fracture elongation of suture reaches the highest point.[21] This is contradictory to the present study as we have found a gradual decrease in the tensile strength of PGA and vicryl post immersion.

The limitations of this study include small sample size and short span of testing for tensile strength post immersion in the extract and artificial saliva. This study can be further continued by analyzing the effect of genistein and nicotine on absorbable sutures in vivo, via animal studies.

Conclusion

From our study we can conclude that the secondary metabolites nicotine and genistein, present in our daily lives, have a significant effect on absorbable sutures such as PGA and vicryl. Genistein has shown to have a good antioxidant activity and hence will help in the healing of the surrounding tissues. Nicotine has a significant anti-inflammatory activity but since it is a carcinogenic substance, it has to be used in a limited amount. Nicotine and genistein also affect the tensile strength and degradation of the sutures. This concludes that sutures can be coated with genistein and nicotine in the future for their antioxidant and anti-inflammatory effect. Future studies need to be conducted to test the coated sutures for their antibiotic properties and their performance in vivo.

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Declarations

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Author contributions

Conceptualization, An.C. and R.E.; Methodology, R.E.; Software, Ad.C.; Validation, R.S.J., Ad.C. and An.C.; Formal Analysis, Ad.C.; Investigation, An.C. and R.E.; Resources, Ad.C.; Data Curation, R.S.J.; Writing – Original Draft Preparation, Ad.C.; Writing – Review & Editing, An.C. and R.S.J.; Visualization, An.C. and R.E.; Supervision, R.S.J.; Project Administration, R.E.; Funding Acquisition, Ad.C.

Conflict of interest

The authors declare that there are no conflicts of interest in the present study.

Data availability

All the data in this study is completely incorporated in the manuscript.

Ethical approval

Not applicable.

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