Original Article

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Dentistry Section

Comparison of Mechanical, Antibacterial and Morphological Properties of Silk Sutures Coated with Silver Nanoparticles and Aloe Vera Herbal Extract: An In-vitro Study

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ABSTRACT

Introduction: Surgical sutures play an important role in wound healing at surgical sites, which are susceptible to microbial infections. These sutures need to prevent bacterial adhesion and proliferation, particularly in areas exposed to oral fluids, to avoid contamination inside the wound. Antibiotic-coated sutures have shown effective antibacterial properties, and silver has emerged as a promising antimicrobial agent. Additionally, Aloe vera, a natural source of bioactive compounds, has been extensively studied for its antibacterial, antiviral, anti-tumour, and anti-inflammatory activities.

Aim: To analyse the morphological, mechanical, and antibacterial properties of plain silk sutures compared to silk sutures coated with silver nanoparticles (AgNPs) and silk sutures coated with AgNPs and aloe vera extract.

Materials and Methods: This in-vitro study was conducted in the White Lab of Saveetha Dental College and Hospital, Chennai, Tamil Nadu, India, over a one-month period from September 2022 to October 2022. The study included three groups, with three samples in each group. Group A consisted of plain silk sutures, Group B consisted of silk sutures coated with AgNPs, and Group C consisted of silk sutures coated with AgNPs and Aloe vera extract. Morphological and microanalytical characterisation was performed using Scanning Electron Microscopy (SEM) images and Energy-Dispersive X-ray Spectroscopy (EDS). Tensile strength was determined using straight-pull and knot-pull tests, following the Instron® method, and knot efficiency. Antibacterial efficacy was evaluated using antimicrobial culture tests for the three groups. Statistical analysis was performed using the Shapiro-Wilk test to assess normality of continuous variables, followed by parametric tests of significance including paired t-tests and Analysis of Variance (ANOVA).

Results: The tensile strength, as determined by the straight-pull test, knot-pull test, and knot efficiency, was highest in Group C, followed by Group B and Group A (statistically significant, p-value <0.001 for straight-pull test, 0.038 for knot-pull test, and 0.002 for knot efficiency). Group B exhibited the highest antibacterial efficacy, followed by Group C, while Group A showed no antibacterial efficacy (statistically significant, p-value <0.001).

Conclusion: This present pilot study suggests that both AgNP-coated and Aloe vera-coated sutures hold promise in preventing Surgical Site Infections (SSI) and promoting wound healing.

Keywords: Scanning electron microscope, Surgical sutures, Wound healing

INTRODUCTION

Surgical sutures play a crucial role in wound healing. They assist in the reapproximation of tissues, promotion of primary healing, and control of haemorrhage. Hence, suture materials must be selected carefully, particularly for sutures used in oral and maxillofacial surgery, which have unique requirements due to the constant presence of saliva, high levels of vascularisation, and functions associated with speech, mastication, and swallowing. In addition to good resistance to traction, dimensional stability, secure knotting, and sufficient flexibility to avoid damage to the oral mucosa, these sutures must also limit bacterial adhesion and proliferation in areas exposed to oral fluids to prevent contamination inside the wound [1,2].

In recent times, antibiotic-coated sutures have demonstrated effective antibacterial efficacy [3-5]. However, the long-term use of antibiotics is known to cause bacterial resistance and increase the virulence of organisms [6,7]. Consequently, researchers are now focusing on the development of new bioactive substances with antimicrobial properties. Silver has emerged as an effective antimicrobial agent, working by attaching to the cell membrane and penetrating inside microorganisms. Silver nanoparticles are particularly effective, targeting the respiratory chain, cell division, and causing cell death [8]. The production of silver nanoparticles through green nano synthetic routes, which utilise biological organisms such as microorganisms and plants, offers environmentally safe, non-toxic, cost-effective, and time-saving applications [9].

Similarly, *Aloe vera*, a natural source of bioactive compounds, has been extensively studied for its biomedical use. *Aloe vera* belongs to the Liliaceae family and has the ability to promote wound healing and treat burns [10,11]. Numerous studies have demonstrated the antibacterial, antiviral, anti-tumour, and anti-inflammatory activity of various parts of *Aloe vera*, including its stem, root, and leaf extracts [12-14]. The chemical composition of *Aloe* has also proven its potential use in cosmetic formulations, food supplements, and medical devices. *Aloe* gel, the clear mucilaginous tissue found in the inner part of *Aloe vera*, contains water and bioactive compounds such as aloin, emodin (anthraquinones), flavonoids, saponin, *Aloe*-mannan, various amino acids, and vitamins. These bioactive compounds significantly contribute to the antibacterial activity of Aloe gel [12,15,16].

While AgNP-coated sutures have been studied for their antibacterial efficacy, the adjunctive effect of Aloe vera extract on these AgNP-coated sutures has not been evaluated in previous studies. This present study aims to analyse the morphological, mechanical, and antibacterial properties of plain silk sutures compared to silk sutures

coated with silver (Ag) nanoparticles and silk sutures coated with AgNPs and *Aloe vera* extract.

MATERIALS AND METHODS

The present in-vitro study was conducted in the White Lab of Saveetha Dental College and Hospital, Chennai, Tamil Nadu, India, over a period of one month from September 2022 to October 2022. The study has been approved by the Institutional Ethical Committee of Meenakshi Ammal Dental College, MAHER, Chennai (MADC/IEC-I/56/2022).

The sample size was not estimated as it is a pilot study, and therefore, three sutures were taken in each group. The groups were as follows:

Group A: Plain silk sutures.

Group B: Silk sutures coated with Silver (Ag) nanoparticles.

Group C: Silk sutures coated with Silver (Ag) nanoparticles and aloe vera extract.

In present study, silver nanoparticles were biologically synthesised using a novel green synthesis methodology from a *Thulasi* extract [17,18]. These nanoparticles were impregnated onto the surface of non-absorbable silk sutures using an in-situ process. Some of these sutures were additionally coated with Aloe vera extract. AgNP and AgNP+*Aloe vera* coated sutures were characterised microanalytically using SEM images and EDS. The tensile strength was determined, and the antimicrobial potential of the sutures was evaluated against certain pathogenic microorganisms.

Study Procedure

Suture materials: Commercially available silk braided black surgical sutures, non absorbable (2 metric, size 3-0) ethicon, supplied by Johnson & Johnson Pvt. Ltd., were used. The suture material was delivered in sterile single peelable foil packages and stored at room temperature.

Preparation of the leaf extract: Thulasi (*Ocimum tenuiflorum*) leaves were selected for the biosynthesis of AgNPs due to cost-effectiveness, ease of availability, and medicinal properties. Fresh and healthy leaves were collected locally, rinsed well, dried, and then cut into small pieces. Next, 10 g of these finely incised leaves were transferred into 250 mL beakers containing 100 mL distilled water and boiled at 80°C for 20 minutes. After cooling, it was filtered, and the filtrate was stored at 4-8°C and used as reducing and stabilising agents in the synthesis of AgNPs [Table/Fig-1] [18].

[Table/Fig-1]: Bio-synthesis of silver nanoparticles using Thulasi extract.

AgNPs synthesis: AgNPs coated suture threads were fabricated using a dip coating method. An aqueous solution of 1 mM silver nitrate ($AgNO_3$) was prepared and used for the synthesis of AgNPs. 10 mL of Thulasi leaf extract was added to 90 mL of the aqueous solution of 1 mM silver nitrate and incubated for six hours. During the synthesis of AgNPs, 12 suture threads (15 cm length) were soaked in the mixture. Complete reduction of $AgNO_3$ to Ag+ ions took place. After six hours, the coated sutures were taken out and dried [Table/Fig-2] [19].

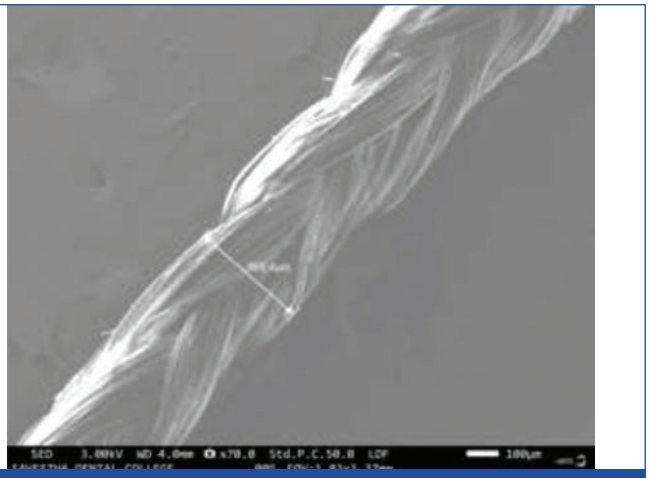
[Table/Fig-2]: Incubation of the coated sutures.

Collection of Aloe vera extract and impregnation of Aloe vera extract on the AgNP coated silk sutures: Fresh Aloe vera plants (Liliaceae family) were collected from local nurseries, and the leaves were washed well with distilled water to remove all contaminants present on the surface. The gel was harvested from the leaves in an autoclaved container and kept at room temperature for further use. The dip coating method was used to coat the sutures. For dip coating, a solution was prepared by mixing 5% Aloe vera gel and 1 g of polyvinyl Alcohol (PVA) in 40 mL of Dimethyl Formamide (DMF). Six AgNPs-coated sutures were dipped in the coating solution for 60 minutes and stirred with a constant rpm of 150 rpm, followed by removal and air drying of the suture for 24 hours [Table/Fig-3] [20].

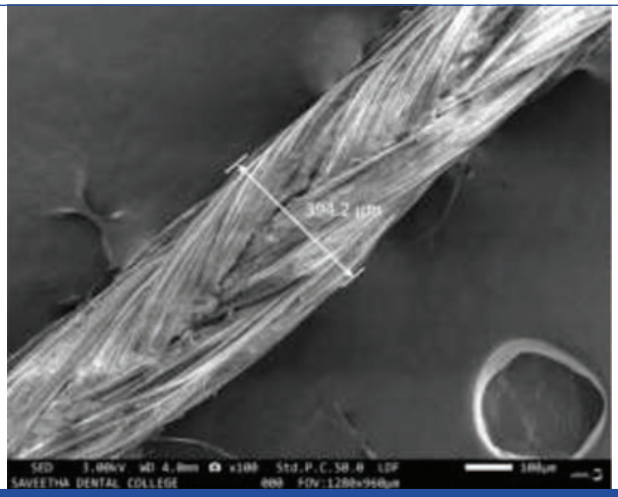
[Table/Fig-3]: Drying of the coated sutures.

Parameters studied: Morphological and Microanalytical characterisation: The surface morphology of the silk fibers and their elemental composition were studied using SEM images and EDS, respectively [Table/Fig-4a-c,5a-b].

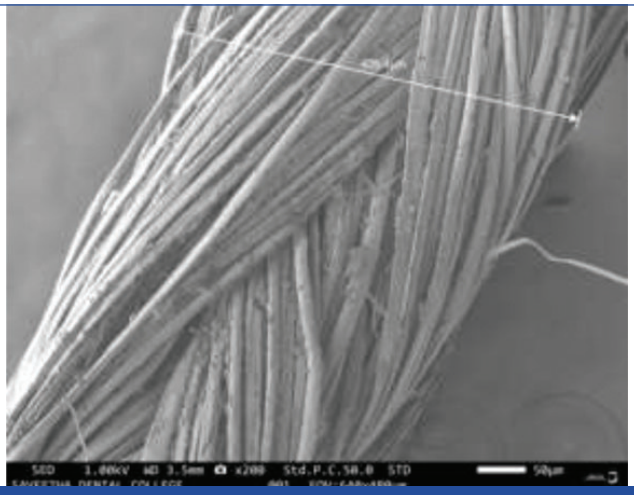
- **Tensile strength:** In order to determine the suture performances in vitro, the sutures were subjected to straight-pull and knot pull tests, adopted from the Instron® method [21,22]. In the straight pull test, the suture material was cut to a length that extends through both grip faces, and the grips were closed with a clamping pressure of 85 psi. Knot efficiency is defined by the loss percentage of tensile strength due to knot tying. It is obtained from the ratio of tensile strength and knot pull strength of the suture (equation (1) [Table/Fig-6a-b] [22]). Knot pull strength (N), Knot efficiency (%)=-X 100 [1] tensile strength (N).
- **Antibacterial efficacy:** The antibacterial activities were checked against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas* colonies of bacteria. Peptone broth inoculum



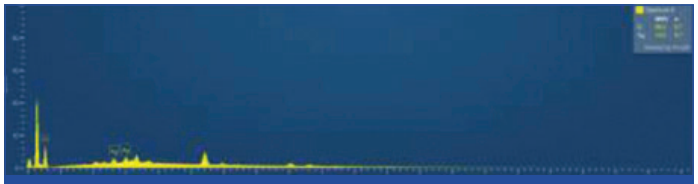
[Table/Fig-4a]: SEM image of Group A=369.4 µm.



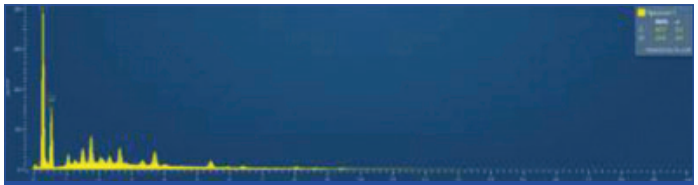
[Table/Fig-4b]: SEM image of Group B=394.2 µm.



[Table/Fig-4c]: SEM image of Group C=426.4 µm.



[Table/Fig-5a]: EDS analysis of Group B.



[Table/Fig-5b]: EDS analysis of Group C.

[Table/Fig-6]: (a) Straight-pull test by the Instron® method; (b) Knot-pull test by the Instron® method.

was prepared, and all microorganisms were inoculated separately and incubated at 37°C for 12 hours. After 12 hours, a 10-8 dilution was taken for culture plating with Mueller Hilton Agar. Then, samples A, B, C were kept at room temperature for two hours and then incubated at 37°C for 12 hours. The zone of inhibition was checked and measured [Table/Fig-7] [23].

[Table/Fig-7]: Zone of inhibition for all three suture groups.

STATISTICAL ANALYSIS

The statistical analysis was done using Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics for Windows, version 23.0, Armonk, NY: IBM Corp. Released 2019) software. The quantitative data were described using mean and standard deviation. All continuous variables were tested for normality using the Shapiro-Wilk test, and the data were found to be normally distributed. Hence, within and between comparisons were made using parametric tests of significance, paired t-test, and ANOVA. (p-value <0.05 was considered statistically significant).

RESULTS

The SEM images and EDS results are reported in [Table/Fig-4a-c,5a,b]. SEM images of all three groups of sutures revealed the typical multifilament structure of the sutures. There was no silver peak in the EDS spectrum of the non-coated suture, nor were there any surface debris on the SEM image of Group A [Table/Fig-4a]. Group B showed silver nanoparticle deposition onto the bio-AgNP-coated sutures, and the presence of the silver ions was detected by EDS analysis [Table/Fig-4b,5a]. Group C showed a higher thickness

of the suture material, which could be due to the gel consistency of the Aloe vera extract. EDS analysis confirmed the Aloe vera coating by the presence of carbon and oxygen atoms [Table/Fig-4c,5b].

Intergroup comparison of mean values of the study parameters:

- **Tensile strength:** In Group A, B, and C, the mean values of tensile strength at break using the Straight pull test were 784.25±161.41, 854.74±57.59, and 873.07±183.53 MPa, respectively; using the knot pull test were 406.08±65.21, 436.95±125.39, and 451.98±200.71 MPa, respectively; and the knot efficiency was 50.05%, 54.67%, and 55.31%, respectively [Table/Fig-8].

Sample	Tensile strength at break (MPa)-Straight pull test	Tensile strength at break (MPa) knot-pull test	Knot efficiency
A	784.25±161.41	406.08±65.21	50.05±0.00
B	854.74±57.79	436.95±125.39	54.67±0.00
C	873.07±183.53	451.98±200.71	55.31±0.00
p-value[A]	<0.001***	0.038*	0.002**

[Table/Fig-8]: Comparison of mean values of the tensile strength among Group A, B and C.
p-value[A]-ANOVA; *p-value <0.05 is statistically significant; **p-value <0.01 is statistically highly significant; ***p-value <0.001 is statistically very highly significant

Tensile strength, in terms of the straight pull test, was greatest for Group C, followed by Group B and Group A. Tensile strength, in terms of the knot pull test, was greatest for Group C, followed by Group B and Group A. Knot efficiency was the highest for Group C, followed by Group B and Group A [Table/Fig-9].

Test	*E.Coli*		*Staph.aureus*		*Pseudomonas aeruginosa*	
Pairwise comparison	Mean difference	p-value	Mean difference	p-value	Mean difference	p-value
A vs B	5.0	0.004**	7.6	0.054**	6.6	0.054**
B vs C	3.0	0.043*	6.3	0.002*	4.5	0.095
A vs C	2.0	0.001***	1.3	0.001***	2.1	0.001***

[Table/Fig-9]: Pairwise comparison of the tensile strength among Group A, B and C.
p-value[A]-Paired-t test; *p-value <0.05 is statistically significant; **p-value <0.01 is statistically highly significant; ***p-value <0.001 is statistically very highly significant

- **Antibacterial efficacy:** In Group A, B, and C, the mean values of *Escherichia coli* were 0, 0.5, and 0.2, respectively; for Staph aureus were 0, 0.766±0.1, and 0.13, respectively; and for *Pseudomonas aeruginosa* were 0, 0.66±0.1, and 0.21, respectively [Table/Fig-10]. Antibacterial efficacy was greatest in Group B, followed by Group C, whereas no antibacterial efficacy was seen in Group A [Table/Fig-11].

Sample	*E.Coli*	*Staph.aureus*	*Pseudomonas aeruginosa*
A	0.0±0.0	0.0±0.0	0.0±0.0
B	5.0±0.0	7.6±0.1	6.6±0.1
C	2.0±0.0	1.3±0.0	2.1±0.0
p-value[A]	<0.001***	<0.001***	<0.001***

[Table/Fig-10]: Comparison of the mean values of the antibacterial efficacy among Group A, B and C.
p-value[A]-ANOVA; *p-value <0.05 is statistically significant; **p-value <0.01 is statistically highly significant; ***p-value <0.001 is statistically very highly significant

Test	Straight pull test		Knot-pull test		Knot efficiency	
Pair-wise comparison	Mean difference	p-value	Mean difference	p-value	Mean difference	p-value
A vs B	70.49	0.004**	30.68	0.054**	4.62	0.054**
B vs C	18.33	0.043*	15.03	0.002*	0.64	0.095
A vs C	88.82	0.001***	45.9	0.001***	5.26	0.001***

[Table/Fig-11]: Pair-wise comparison of the antibacterial efficacy among Group A, B and C.
p-value[A]-ANOVA; *p-value <0.05 is statistically significant; **p-value <0.01 is statistically highly significant; ***p-value <0.001 is statistically very highly significant

DISCUSSION

The SEM and EDS analysis were mainly done to confirm the uniform coating of the tested elements, namely the AgNP and the Aloe vera extract, on the suture material. The results thereby confirmed the same. Similar results were also obtained by De Simone S et al., and Gallo AL et al., who coated the silk and polyglactin 910 PGLA sutures with silver using a process based on the photoreduction of silver solution, respectively [24,25]. Good tensile strength is important as it ensures the prevention of knot slippage and breakage, clot stability, and protection of the wound site for 14 days while the majority of the reconstruction takes place during the healing period [26]. In Periodontics, it is significant for regenerative flap surgeries as well as mucogingival surgeries. In present study, tensile strength results showed that Group A < B < C, with the greatest in the AgNP+*Aloe vera* vera group. Similar results were obtained by Dhas SP et al., found that functionalised silk fibers had better breaking strength due to the adsorption of biofunctionalised AgNPs onto the silk fibers [27].

In another study by Ravindra S et al., cotton fibers impregnated with AgNPs showed enhanced mechanical properties due to the binding of AgNPs onto the hydroxyl groups of the cellulose chains of cotton fibers [28]. In contrast to our study, Dhafer CeB et al., in a mechanical study of polypropylene suture with Ag nanocomposite, showed that the grafting of the AgNPs on the polypropylene surface had no effect on the suture strength [22]. In present study, bio-synthesised AgNP along with an additional coating of Aloe vera led to a uniform coating of the sutures, which increased the binding and tensile strength of the sutures.

The antimicrobial property of AgNP comes from its activity against Reactive Oxygen Species (ROS) formation, protein-AgNP interaction, inhibition of Deoxyribonucleic acid (DNA) replication, and disruption of microbial cell walls [29-31]. Zhang S et al., and Baygar T et al., showed that AgNP-coated silk sutures had strong antimicrobial activity against *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*, which is in accordance with our study [32,33]. Ghafoor B et al., showed effective bactericidal properties against *E.coli* and P. aeruginosa of Aloe vera gel on braided black silk sutures [20]. However, in our study, Group B showed better antibacterial efficacy than Group C [Table/Fig-7]. Limited antibacterial efficacy of aloe vera was observed within 24 hours of incubation, which could be due to the potential blockage of the AgNP's antibacterial action by the additional layer of aloe vera gel. This may have prevented a synergistic effect of AgNP+Aloe vera from being observed, as hypothesised. However, Tippayawat P et al., showed significant antibacterial efficacy against *S.epidermis* and *P.aeruginosa* with AgNP+Aloe vera, but it is important to note that they used a novel hydrothermal process of grafting AgNP+Aloe vera together [34]. Further evaluation with longer incubation periods and different loading techniques of AgNP+Aloe vera may show increased efficacy. Future research prospects could include evaluating the optimum level of AgNP and Aloe vera needed for the best efficacy, studying if, monofilament sutures can be coated with AgNPs or Aloe vera, and studying the in-vivo anti-inflammatory efficacy of both coated sutures.

Limitation(s)

The short incubation period of the aloe vera coating and the small sample size. The study did not include the culture of gram-negative periodontal pathogens, which could have provided more insight into its usage in periodontal surgeries.

CONCLUSION(S)

As a pilot study, the tensile strength and antimicrobial efficacy of the coated sutures were significantly higher than the uncoated suture. Both AgNP and Aloe vera coated sutures appear to be promising candidates for preventing surgical site infections and aiding in wound healing. Further research with extended time periods and new

approaches to coating aloe vera and AgNP may provide a better understanding of the characteristics of these coated sutures.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 29, 2022
- Manual Googling: Feb 21, 2023
- iThenticate Software: Jun 20, 2023 (14%)

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EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

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