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# Development of herbal drug loaded antimicrobial silk suture

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Drug loaded antimicrobial silk suture have been developed for use in both wound closure and wound healing with an intention to prevent surgical site infections. The material has been analyzed for various suture properties such as tensile strength, elongation, knot strength, bending properties and diameter. The suture material is coated with chitosan (biopolymer) and incorporated with herbal drug. Cold maceration technique is used to extract *Cynodon dactylon* drug for the study. SEM reveals the uniform deposition of chitosan on to the surface of the suture material. The polymer and drug coated samples are subjected to FTIR analysis. To optimize the process parameter, response surface method is adopted using Box-Behnken experimental design. The drug loaded samples are also subjected to antimicrobial study (AATCC 100) against *S.aureus* and *E.coli* and found to have effective bacterial reduction percentage. Thus, the natural drug loaded suture material would be a better alternative for synthetic drug loaded suture which is found to have some demerits.

**Keywords**: Antimicrobial property, Biopolymer, Chitosan, Herbal drug, Silk, Suture

## 1 Introduction

Sutures are natural or synthetic materials available in monofilament, multifilament, twisted and braided forms, used widely in wound closure, ligates injured blood vessels and to draw tissues together[1, 2]. They are classified into absorbable and non-absorbable category and attached with a metallic needle at one of the fibrous ends. The most important properties of suture materials are physical, mechanical, handling, biological and biodegradation properties. All these properties are interrelated[3,4].

Sutures are found to be a major cause for surgical site infections (SSI), occuring within 30 days after a surgical operation (or within one year if an implant is left in place after procedure) which affects either the incision or deep tissues at the operation site[5]. Suture, being a foreign material in a surgical wounds, enhances the susceptibility of surrounding tissues of wound to infections[6]. Bacteria present in the surgical wound not only contaminate the tissues but also the suture material, which leads ineffectiveness in decontaminating the wound[7]. Bacterial attachments and colonization occurs in all suture material and leads to surgical site infections[8]. Implants have non-shedding surface, skin or other bacteria to form an extra cellular matrix (biofilms), protecting the bacteria from host defense factors[9,10]. These biofilms bacteria are difficult to treat and they are less sensitive to antibiotics and antiseptics. It is necessary to remove the implant, and antibiotic treatment is essential if biofilms infection is formed[11]. In order to overcome this, many antimicrobial sutures were developed by incorporating suitable antibiotics, antiseptics or their combination. Triclosan, commonly used in in antimicrobial sutures, was found to have some demerits such as prematurely change of tadpoles into frogs and reduced sperm production in male rats [12].

Silk is a natural biomaterial which consists of fibrion. It helps in cell attachment and proliferation. Silk suture also possesses good knot tyeing quality[13,14].

Chitosan is considered as one of the most valuable polymers for biomedical and pharmaceutical applications due to its biodegradability, biocompatibility, non-toxicity, antimicrobial property, non-toxicity, and anti-tumor properties[15,16]. Chitosan is a benefit to wound healing because it stimulates hemostasis and accelerates tissue generation[17]. Suture materials are normally coated with silicon or wax was found to create inflammatory and thrombotic response to the tissuess[18]. Coating with chitosan prevents inflammation as well as scar formation with and provides antimicrobial property since silk is easily prone to microbial infection[19].

Aqeous extract of *Cynodon dactylon* (a traditional herb), commonly called as Bermuda grass was evaluated for their antioxidant, anti-inflammatory

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action, while its fresh juice has shown the immunomodulatory and DNA protective activity[20-22]. The aerial part extract of *Cynodon dactylon* was found to possess alkaloids, phenols, tannins and flavonoids on preliminary screening. An antibacterial effect and wound healing property of the grass was investigated by many researchers[23-27].

The main aim of the present study is to develop an antimicrobial silk suture preventing SSI by using natural materials having rich medicinal values, which includes biomaterials and medicinal plants[27].

## 2 Materials and Methods

### 2.1 Suture Material

Silk filament (20 denier) procured from Sarvodhya Sangam, Coimbatore was used in the study. Chitosan (medium molecular weight) was procured from Sigma Aldrich Pvt. Ltd., Bangalore. All the chemicals used in the study were of analytical grade.

### 2.2 Degumming

Silk filaments were treated with $Na_2CO_3$ at 100°C for 30 min to remove sericin a natural gum present in silk. After degumming, rinsing was done with warm water, subsequently the skein is neutralized with mild citric acid and 10% weight loss is reported.

### 2.3 Braiding

The silk filaments were braided using SEMCO circular braiding machine of 12 carrier arrangement. A regular (1/1) braid pattern was constructed with multifilament yarns.

### 2.4 Measurement of Suture Properties

Diameter was measured by using projection microscope at five different positions. Average of 20 observations was made. Tensile strength, knot strength and breaking strength were measured using Instron-5500R (ASTM D2256) tensile strength tester. The tests were carried out at the gauge length of 5cm and test speed of 100 mm/min. For knot strength simple surgeons knot was tied in the middle of the suture. Bending rigidity and bending hysteresis of the suture material was tested on KES-Fb2 bending tester.

### 2.5 Plant Material

#### *2.5.1 Collection and Authentication of Plant*

The herb *Cynodon dactylon was* collected from the local habitat of Coimbatore in area free from pesticides. The herb was identified and authenticated by Dr M Palanisamy of Botanical Survey of India, TNAU Campus, Coimbatore. Voucher specimen (No. BAI/SRC/5/23/2013-14/Tech/1765) was deposited in the herbarium.

#### *2.5.2 Extraction*

The grass was washed thoroughly, shadow dried, powdered and extracted with ethanol by cold maceration technique. In this method, powdered *Cynodon dactylon* was blended with ethanol for 2 days with agitation at room temperature. The extracts were then filtered by using filter paper and concentrated using a rotary evaporator at 40°C under reduced pressure. Finally, the extracts were weighted and stored at -20°C till they are used [28].

#### *2.5.3 Application of Polymer and Drug*

Chitosan solution was prepared in 1% lactic acid by stirring in a magnetic stirrer for 1 h at 60°C. The material was immersed in the solution for 24 h. The sample was then washed with deionized water, subsequently padded and then dried at 60°C for 5 min. The sample was then incorporated with drug by immersing the material in the drug solution for 24 h and then dried at 60°C.

### 2.6 Characterization Studies

The surface characteristic of the silk suture was studied using scanning electron microscope.

The polymer and drug coated samples were subjected to FTIR analysis. SHIMADZU spectrophotometer was used for the analysis.

### 2.7 Antimicrobial Assay

The antimicrobial activities of the treated suture material and the control (untreated suture) material were tested against *Staphylococcus aureus* (MTCC96) a Gram positive organism and *Escherichia coli* (MTCC724) a Gram negative organism using quantitative test method (AATCC 100) with standard procedure cited in the literature[29].

### 2.8 Statistical Analysis

Response surface optimization process using Box-Behnken experimental design was adopted in the study for optimization of the process as reported cited in the literature[30]. The response surface equation for each independent variables was obtained by using the software package MINITAB 15. The suture was coated with chitosan using three different concentrations (1, 1.5 and 2%) and kept for 24 h.

The sample was then washed with deionized water and padded followed by drying at 60°C for 5 min. The polymer coated sample was immersed in the extract with three different concentrations (3, 5 and 7%) and allowed for 24 h followed by padding, and dried at three different temperatures (60, 80 and 100°C) for 5 min. Thus, total 15 tests were carried out. Variables like concentration of the polymer, concentration of the extract, drying temperature were taken as independent variable $X_1$, $X_2$, $X_3$ respectively. The low, middle and high levels of variables were designated as +1, 0, and -1 respectively (Table 1). The dependent variable used in the study was antimicrobial efficacy. The design of experiment is given in Table 2. The calculation was carried out using multiple regression analysis, using the equation as given below:

$$Y= C_0+C_1X_1+C_2X_2+C_3X_3+C_{12}X_1X_2+C_{13}X_1X_3+C_{23}X_2X_3+C_{11}X_1^2+C_{22}X_2^2+C_{33}X_3^2$$

where $Y$ is the response variable (dependent variable); $X_1$, $X_2$, $X_3$ are predictor variables (independent variable); $C_0$ is constant; $C_1$, $C_2$, $C_3$ are linear co-efficients; $C_{12}$, $C_{13}$, $C_{23}$ are cross product co-efficient; and $C_{11}$, $C_{22}$, $C_{33}$ are quadratic co-efficient.

## 3 Results and Discussion

### 3.1 Evaluation of Suture Properties

The braided multifilament suture material was evaluated for its properties before and after coating (Table 3). Tensile properties are important for a suture in making a knot. Sutures may easily break if tensile strength is higher than the knotting force. In both the samples, tensile strength is found to be more than the knot strength. This observation well correlates with previous findings, stating that the knot being the weakest part when subjected to tension[31, 32]. It is observed that the coated suture exhibits high tenacity which may be due to better binding of fibres by chitosan, thereby resisting the axial load. The results are found consistent with previous works[33,34].

Table 1 — Level of variables used for the trial

| Property | Levels | | |
|---|---|---|---|
| | -1 | 0 | +1 |
| Concentration of the polymer ($X_1$), % | 1 | 1.5 | 2 |
| Concentration of the extract ($X_2$), % | 3 | 5 | 7 |
| Temperature ($X_3$), °C | 60 | 80 | 100 |

Table 2 — Box-Behnken three level-three-variable experimental design

| Test No. | Levels | | |
|---|---|---|---|
| | -1 | 0 | +1 |
| 1 | 1 | 3 | 80 |
| 2 | 2 | 3 | 80 |
| 3 | 1 | 7 | 80 |
| 4 | 2 | 7 | 80 |
| 5 | 1 | 5 | 60 |
| 6 | 2 | 5 | 60 |
| 7 | 1 | 5 | 100 |
| 8 | 2 | 5 | 100 |
| 9 | 1.5 | 3 | 60 |
| 10 | 1.5 | 7 | 60 |
| 11 | 1.5 | 3 | 100 |
| 12 | 1.5 | 7 | 100 |
| 13 | 1.5 | 5 | 80 |
| 14 | 1.5 | 5 | 80 |
| 15 | 1.5 | 5 | 80 |

### 3.2 Scanning Electron Microscope

SEM images of chitosan coated and uncoated suture materials are shown in Fig.1. SEM reveals the uniform deposition of chitosan on the surface of the suture material.

### 3.3 FTIR Analysis

FTIR spectra for chitosan coated sample are obtained in the range of 800 - 4000 cm$^{-1}$. The broad band occured at 3286 cm$^{-1}$ confirms the presence of C-OH group. A sharp band occured at 2924 cm$^{-1}$ indicates the presence of C-H group; (C=O) - stretching is observed in the region of 1735 cm$^{-1}$ which confirms the presence of ester group; and (C-$NH_2$) - stretching is observed in the region of 1519 -1627 cm$^{-1}$. It is observed that the band at 1018 cm$^{-1}$ in chitosan polymer is shifted towards 1041 cm$^{-1}$ in the drug incorporated sample.

Table 3 — Suture properties

| Property | Before coating | After coating |
|---|---|---|
| Tenacity, cN/tex | 35.48 | 36.18 |
| Elongation, % | 20.03 | 19.8 |
| Knot strength, cN/tex | 24.17 | 24.65 |
| Elongation with knot, % | 10.73 | 10.25 |
| Diameter, mm | 0.48 | 0.484 |
| Bending rigidity g.cm$^2$/yarn | 0.0997 | 0.1081 |
| Bending hysteresis gf.cm/yarn | 0.1126 | 0.1768 |
| Breaking strength, gf | 3540.17 | 3678 |

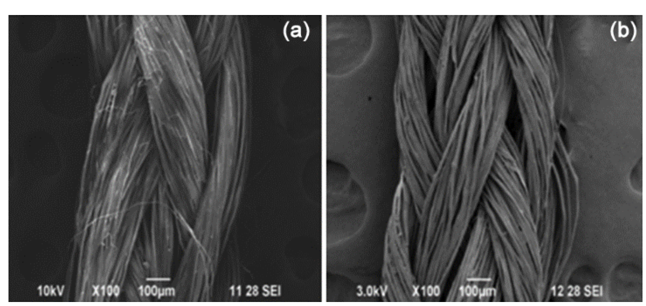


Fig.1 — SEM images of (a) coated sample and (b) uncoated sample

**3.4 Evaluation of Antimicrobial Efficacy**

The silk suture materials have been tested for bacterial reduction using shake flask method against *S.aureus* and *E.coli*. Bacterial reduction percentage for 15 test samples are shown in the Table 4. Bacterial reduction is found to be good in the samples with higher extarct concentration. In all the samples, the bacterial reduction is found to be more in *E.coli* than in *S.aureus*, which is quite controversy because usually Gram negative bacteria exhibit more resistance than Gram positive bacteria. This may be due to the reason that the chitosan being positively charged, interaction with negatively charged residues will be more at the cell surface of many fungi and bacteria which are responsible for the altertion of cell permeability, resulting in the leakage of intracellular substances[35,36]. This result also matches with the previous study made on antibacterial activity of *Cynodon dactylon,* where the active substance saponins is found to be effective against Gram negtaive bacteria[37].

**3.5 Process Optimisation Results**

The influence of three variables at three levels has been investigated using Box-Behnken experimental design. The regression equation obtained gives the antimicrobial efficacy of the suture material against *S.aureus* and *E.coli* as the function of different polymer concentration, drug concentration and drying temperature. Regression equation for the responses are given in Table 5 along with F-ratio and p-value. $R^2$ represents the proportion that model can explainfor the variation in the response. Models with $R^2$ value greater than 60% are considered to be a valid model[38]. The value of 97.38% of $R^2$ in case of *S.aureus* indicates that the model developed explains 97.37% of the variance in the dependent variable. $R^2$ (adj) of 92.67% indicates that 5% error can occur in the model. The same applies for *E.coli*.

Table 4 — Efficacy of treated samples based on Box-Benkhen experimental antimicrobial design

| Test No. | Bacterial reduction, % | |
|---|---|---|
| | *S.aureus* | *E.coli* |
| 1 | 70.84 | 72.1 |
| 2 | 76.4 | 81.2 |
| 3 | 87.1 | 89 |
| 4 | 97.2 | 98.4 |
| 5 | 79.3 | 81.4 |
| 6 | 88.9 | 91 |
| 7 | 79.1 | 80.8 |
| 8 | 96.1 | 95.7 |
| 9 | 86.5 | 89 |
| 10 | 93.4 | 94.6 |
| 11 | 85 | 87 |
| 12 | 93.8 | 95.1 |
| 13 | 92.5 | 93 |
| 14 | 92.5 | 93 |
| 15 | 92.5 | 93 |

If F ratio is larger, the average amount of variation between groups will be greater than that of within groups and vice-versa. The effects are found statistically significant when p-value, defined as smallest level of rejection, is less than 0.05. Smaller the *p*-value, the higher is the significance of the corresponding coefficient.

It is observed that there is increase in bacterial reduction with the polymer concentration only upto 80-85%. Maximum reduction of >95% is observed with the increase in extract concentration. The temperature is found to have no influence on the bacterial reduction.

Bacterial reduction of 80-85% is attained at maximum polymer concentration. Maximum reduction of bacteria (>95%) is observed at maximum extract concentration.

The negative coefficient of a variable in response surface equation indicates that particular characteristics decreases with increase in that variable, while positive coefficient indicates the reverse trend[39].

It is inferred that the suture treated with 1.7% chitosan and 7% drug at 60°C exhibits optimum antimicrobial efficacy. Trial has been conducted with the optimised process parameters and the antimicrobial efficacy of the treated sample is compared with the untreated sample by bacterial reduction test at the time intervals of 0 h and 24 h.

It is inferred that the untreated sample has no effect even after 24 h, but the treated sample achieves 95% and 97% reduction of *S.aureus and E.coli* after 24 h respectively. The images of bacterial colonies of treated sample (0h, 24 h) of *S.aureus* and *E.coli* are shown in Fig. 2.

Table 5 — Regression equations for the responses

| Equation | R | $R^2$ | F ratio | P-value |
|---|---|---|---|---|
| $Y$=-26.96+64.75$X_1$+10.488$X_2$+ 0.613$X_3$-19.7$X_1^2$-0.420$X_2^2$- 0.002$X_3^2$+0.33$X_1X_2$+ 0.0325$X_1X_3$-0.04$X_2X_3$ | 97.38 | 92.67 | 20.67 | 0.002 |
| $Z$=1.603+61.6$X_1$+8.42$X_2$+ 0.137$X_3$-18.45$X_1^2$-0.303$X_2^2$- 2.18$X_3^2$+0.075$X_1X_2$+0.04$X_1X_3$- 0.027$X_2X_3$ | 95.3 | 86.83 | 11.25 | 0.008 |

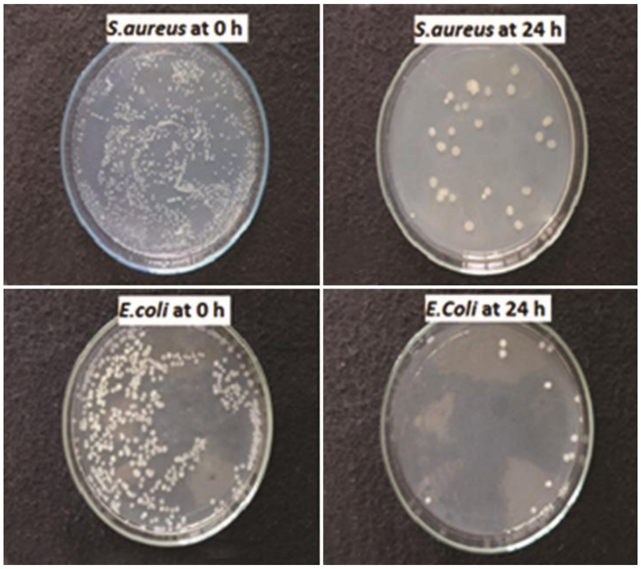


Fig. 2 — Bacterial colonies of treated sample at 0 h and 24 h

## 4 Conclusion

In this study silk has been used as suture material and fabricated using a circular braiding machine. The silk suture is coated with chitosan (a biopolymer) and incorporated with *Cynodan dactylon* (a natural drug). The coated suture is found to have good properties. In order to obtain optimum antimicrobial efficacy, response surface optimization process using Box-Behnken experimental design is applied. It is found that 1.7% chitosan and 7% drug at 60°C has optimum antimicrobial efficacy with effective bacterial reduction percentage against *S.aureus* and *E.coli*, when subjected to antimicrobial study.

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