

Development of Antibacterial Silk Sutures Using Natural Fungal Extract for Healthcare Applications

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Abstract

The purpose of the study was to treat silk sutures with natural fungal pigment namely *Thermomyces* of different concentrations 1.5%, 2.0% and 2.5%, and to analyse its effect on the properties of silk sutures such as tenacity, knot strength, friction and antimicrobial activity. The result showed that the pigment concentration in the selected range has no significant effect on friction, tenacity and knot strength of silk sutures. Antimicrobial test results showed that as the pigment concentration increases the antimicrobial activity also increases against both *E. coli* and *S. aureus* bacteria. At 2.5% concentration, a zone of inhibition of 10 mm and 14 mm are observed against *E. coli* and *S. aureus* respectively. Silk suture treated with optimum concentration of the natural fungal pigment is appropriate to retard the exponential growth of *S. aureus*, a gram-positive bacterium and *E. coli* a gram-negative bacterium and hence silk sutures can be developed with the required characteristics for healthcare applications.

Keywords: Pigment; Fungal; Antimicrobial; Bacterium

Introduction

Wound closure using suture materials is an integral part of the surgical process. Sutures are natural or synthetic textile biomaterials widely used in wound closure, to ligate blood vessels and to draw tissues together [1]. Sutures consist of a fibre or fibrous structure with a metallic needle attached at one of the fibre ends and they can be classified into two broad categories namely absorbable and non-absorbable sutures. The most crucial requirements of suture materials are physical and mechanical properties, handling properties, biocompatibility, and antimicrobial nature [2]. Till date, there is no single suture material which can fulfill all the crucial requirements of sutures [3]. The present surgeon has several choices of suture material available and he may choose them based on availability and his familiarity.

Silk, a natural non-absorbable suture material has been used as biomedical suture for centuries due to its advantageous characteristics. However, one of the major problems associated with the silk is its poor microbe resistance characteristics. Several researchers have used different antimicrobial agents onto silk sutures to impart microbe resistance characteristics. Researchers have also used silver doped bioactive glass powder to coat silk surgical suture [4]. Recently, studies on the effect of chitosan coating on the characteristics of silk sutures [5]. Another study on tetracycline coating on silk sutures was carried out and they investigated the effect of tetracycline treatment on silk suture properties [6].

Recently, antimicrobial finishing of textiles using microbial dyes have received greater attention as they require less labour, land, and cost effective solvents for extraction as opposed to higher plant materials. In this study, silk sutures are treated with *Thermomyces*, a natural fungal extract and its effect on the properties of silk sutures such as antimicrobial activity, friction, tenacity and knot strength are studied.

Materials and Methods

Materials

Bombyx mori silk filaments were used for the study. All other chemicals were analytical grade and used as received.

Methods

The process of development of silk suture is shown in the Figure 1. The following section discusses each step in detail.

Isolation of silk fibroin

The raw silk filaments were immersed in an aqueous solution of 0.1% (w/v) sodium carbonate at 98°C - 100°C for 30 minutes, to remove sericin [7]. The sericin free silk filaments were subsequently washed with copious water to remove sodium carbonate. The silk filaments were subsequently dried and conditioned at an atmosphere of 27°C and 65% relative humidity for 48 hours.

Extraction of fungal pigment from *Thermomyces*

Czapek yeast broth was prepared and *Thermomyces* culture was inoculated and incubated as stationary cultures for 5 days. After incubation the grown up culture was filtered to separate the fungal biomass from the broth. The supernatant broth was filtered using a sterilized muslin cloth to remove the fungal mat. To the filtrate, one volume of 95% (v/v) methanol was added and the mixture was kept on a rotary shaker for 30 min at 150 rpm and at 35°C. The mixture was then centrifuged at 5000 rpm for 15 min. The same process was repeated for removal of fungal biomass and the filtrate was filtered through a preweighed 47 mm filter paper. Next, the absorption spectrum was observed at 300-600 nm using spectrometer (U-2000, Hitachi Ltd., Tokyo, Japan.) The purified pigments were kept in a Buchi rotary evaporator and lyophilized (Lyobeta 35) to obtain the yellow pigment in a powder form [8]. The sample of the extracted pigment is shown in Figure 2.

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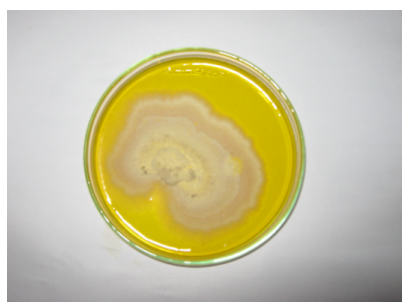
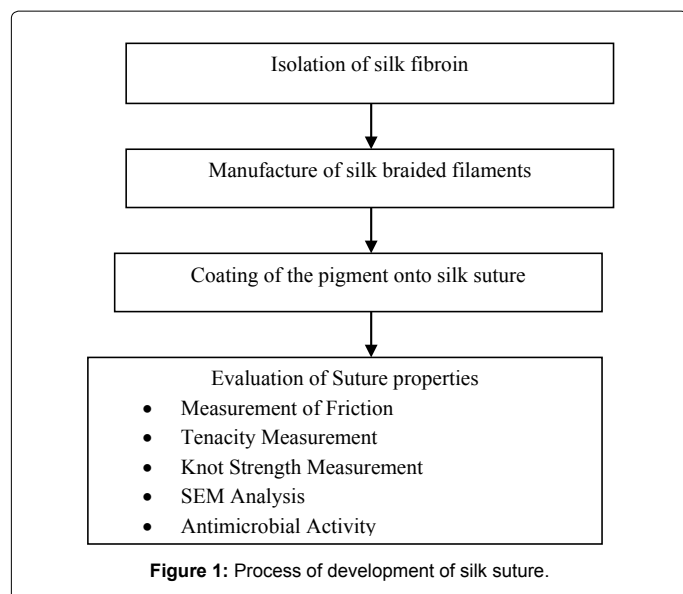


Figure 2: Sample of extracted pigment.

Manufacture of braided silk filaments

Braided silk filaments were manufactured using 16 sericin free silk yarns through a circular braiding machine with 16 carrier arrangement. The count of the braided silk suture was 124 tex.

Extraction of *Thermomyces* and coating onto silk braided filaments

Pigments from *Thermomyces* of different concentrations 1.5%, 2.0% 2.5% (w/v) were prepared as per the standard procedure. The silk filaments were then immersed in pigments of different concentrations for 24 hours at room temperature. The silk sutures were then dried at room temperature for 24 hours.

Friction measurement

The frictional properties of braided silk sutures were measured using Lawson Hemphill Dynamic Friction Tester. The treated and untreated braided silk sutures were tested for friction at a constant sliding speed of 150 m/min and at an input tension of 60 cN. For all the friction testing, 180 degree wrap angle was maintained and for each set of experiment 20 tests were conducted.

Tenacity and knot strength measurement

Tensile Tester (Instron Make) was used to measure the tenacity and knot strength of silk sutures. The braided silk sutures were tested

for tenacity and knot strength at a gauge length of 150 mm and extension rate of 90 mm/min. In the knot strength measurement, knot was formed with square knot method. For each test method at least 20 readings were taken.

SEM analysis

The surface characteristics of silk filaments were studied using Scanning Electron Microscope (JSM 6390) after coating them with pigment.

Antimicrobial activity evaluation

The *Thermomyces* treated and untreated silk filaments were evaluated for their antimicrobial activity using Agar Diffusion Test (SN 195920- 1992) and Shake Flask Method (AATCC 100). All the tests were carried out in triplicate and the results were averaged.

Statistics

Datas were expressed as mean \pm standard deviation. Statistical analysis was carried out using F- test. A value of $P < 0.05$ was considered to be statistically significant.

Results and Discussions

Effect of natural fungal treatment on tenacity and knot strength

Tenacity of suture material is significant for the practitioner making a knot. If the material is too weak and the knotting force is stronger than tensile strength of suture material, suture can easily break while tightening the knot. Therefore it is essential to know the tensile properties of sutures [9]. The Figures 3 and 4 show the effect of pigment concentration on tenacity and knot strength of silk sutures respectively. From the Figures 3 and 4 it is observed that the pigment treated and untreated silk sutures exhibits similar tenacity and knot strength and there is no significant difference between the properties of

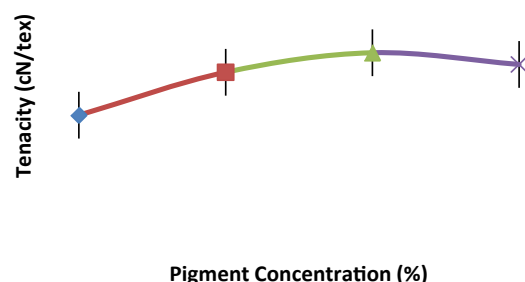


Figure 3: Effect of pigment concentration on tenacity.

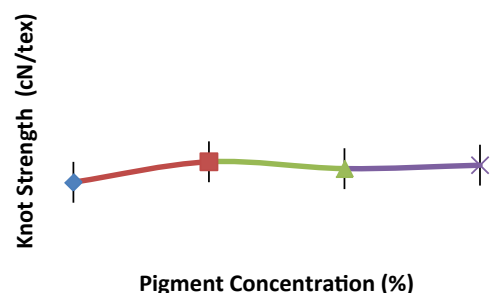


Figure 4: Effect of pigment concentration on knot strength.

treated and untreated samples. This shows that the incorporation of the pigment into the silk sutures has no significant effect on both tenacity and knot strength.

Furthermore, it is observed that for the untreated and all the pigment treated sutures, the knot strength is lower than the tenacity as shown in Figures 3 and 4. The failure of strands occurred at the knot rather than along the suture strand, indicating the knot itself causes an area of high stress concentration [10]. Several factors may contribute to failure occurring at the knot rather than along the suture [11]. First, breakage at the knot may be caused by forces being oriented at the knot at an acute angle to the suture axis. Second, the suture yarn in the knot region may be weakened during knot construction and during loading [12]. Third, tightening of the knot and the friction between yarns in the knot may contribute to the failure [13].

Effect of natural fungal treatment on friction

Braided silk structures are difficult to pass through tissue because of tissue drag and thus they cause a greater extent of tissue damage. Also, braided sutures provide higher frictional values than the monofilament sutures [14]. Hence braided sutures are given special surface coatings to reduce friction. The common form of characterizing the frictional properties of yarns and filaments is the coefficient of friction [15]. The frictional properties of pigment treated silk sutures are shown in the Figure 5. From Figure 5, it is observed that the pigment concentration in the selected range has no significant effects on frictional force. The results suggest that pigment treated silk suture does not generate more friction than the untreated silk sutures.

Effect of natural fungal treatment on antibacterial activity

Bacterial species are capable of colonizing different surfaces and proliferating on them, forming adherent biofilms. This could represent a major problem for implantable suture material. Experimental and clinical data indicate most of the wound infections begin around material left within the wound, and that the incidences of post-surgical complications are directly related to the degree of contamination at the time of material placement. Measuring for reducing the risk of surgical site infections include surgical technique, appropriate antimicrobial agent, adjunctive strategies for reducing wound contamination and promoting wound healing [16].

In this study two different types of bacteria are selected namely *S. aureus*, a gram-positive bacterium and *E. coli* a gram-negative bacterium. *S. aureus* causes skin and tissue infections, septicemia, endocarditis and meningitis whereas *E. coli* causes urinary tract and wound infections [17]. The antimicrobial activity of pigment treated silk sutures tested using agar diffusion method is given in the Figures 6 and 7.

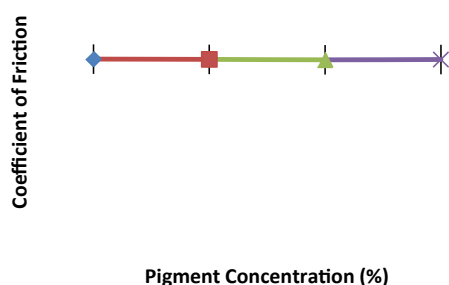


Figure 5: Effect of pigment concentration on friction coefficient.

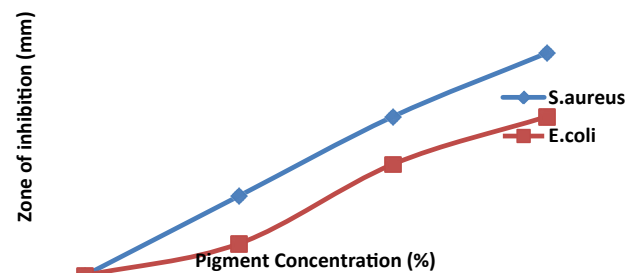


Figure 6: Zone of inhibition as a function of pigment concentration.



(a) *S. aureus*



(b) *E. coli*

Figure 7: Antibacterial activity of the fungal treated silk suture against *S. aureus* and *E. coli*.

From the Figures 6 and 7, it can be seen that silk sutures treated with pigment exhibit excellent antimicrobial activity against both *E. coli* and *S. aureus*. This may be due to the intrinsic antimicrobial characteristics of the pigment. The most accepted mechanism for microbial inhibition by fungal pigment is the presence of anthraquinone compounds, carboxylic acids and pre-anthraquinones [18]. The results also showed that pigment treated silk suture exhibits lower antimicrobial activity against gram negative bacteria *E. coli* than gram positive bacteria *S. aureus*. These results are correlated with the data published previously by the researchers. Moreover, the size of zone is higher for the suture with higher pigment concentration%, which is essential to prevent infectious diseases.

Morphology of pigment free and pigment loaded silk sutures

The SEM micrographs of the pigment free and 2% pigment loaded silk sutures are shown in Figures 8 and 9 respectively. From Figure 9, it is clear that pigment particles are well deposited on the surface of braided silk suture.

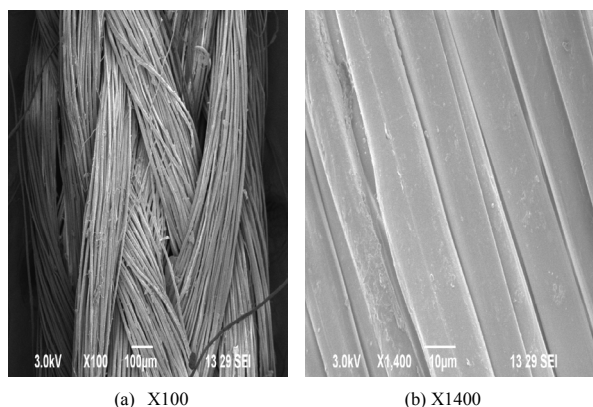


Figure 8: SEM micrograph of the pigment free braided silk sutures.

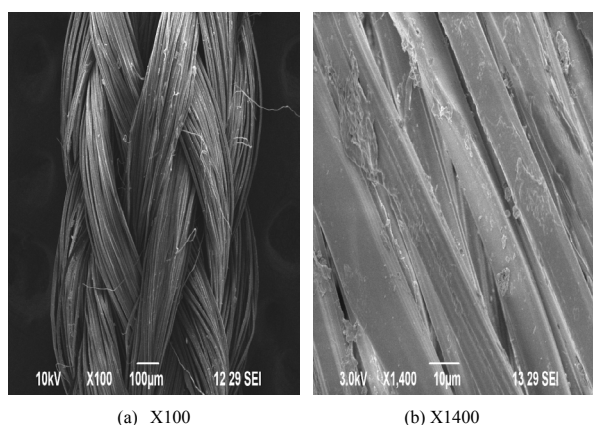


Figure 9: SEM micrograph of the 2 % pigment loaded braided silk sutures.

Conclusions

Silk suture produced was treated with natural fungal extract at optimum concentration and the effects of natural fungal treatment on the suture properties were studied. The result showed that the tenacity and knot strength of silk braided sutures increased compared to the untreated silk suture. The frictional properties of both the fungal treated silk suture and the untreated silk suture were determined by the dynamic coefficient of friction and there is a slight reduction in frictional value found in the treated silk suture compared to the untreated silk suture. The uniform deposition of natural fungal pigment on to the surface of the silk braided suture was confirmed by Scanning Electron Microscopy. The antibacterial activity of fungal treated silk braided suture at optimum concentration against *S. aureus* and *E. coli* is found to be good compared to the untreated silk suture. The result suggests that the silk suture treated with optimum concentration of the natural fungal pigment is appropriate to retard the exponential growth

of *S. aureus*, a gram-positive bacterium and *E. coli* a gram-negative bacterium and hence silk sutures can be developed with the required characteristics for healthcare applications.

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