CHAPTER 6

DEVELOPMENT OF ANTIMICROBIAL SILK SUTURES: CHITOSAN TREATMENT AND CHARACTERIZATION

6.1 INTRODUCTION

Silk from the silkworm, *Bombyx mori*, has been used as biomedical suture material for centuries. This reflects the high biocompatibility of silk, despite silk being a foreign protein to mammals. As a substrate, silk protein is good for mammalian cell adhesion and proliferation. Recent studies reported the use of silk in oral administration (Vepari et al 2007). The excellent biocompatibility and functionality of silk has led to the development of various biomedical devices (Seok et al 2009). As a suture, silk is still popular in ocular, neural and cardiovascular surgery, but has also been used in a variety of other tissues in the body. Silk’s knot strength, frictional characteristics and ability to lay low to the tissue surface make it a popular suture in cardiovascular applications where bland tissue reactions are desirable for the coherence of the sutured structures (Dumitriu 2002).

In order to enhance the material properties and reduce fraying, the silk sutures are usually coated with wax or silicone. Conversely, coated sutures may provoke inflammatory tissue reactions if the pieces of the coating flake off and migrate into surrounding tissues. To reduce this problem, coating should have an affinity for suture filaments. Altman et al (2003)
reported that the elimination of wax coating significantly diminished the initially high thrombotic response to silk. Therefore, an innovative coating substance is needed to improve the frictional characteristics of silk sutures.

Furthermore, silk is a natural protein fibre and easily prone to microbial infection (Liu et al. 2010). The use of antimicrobial sutures is expected to provide protection from wound infection. Several approaches have been reported in developing antimicrobial sutures. Recently, the incorporation of chitosan (Figure 6.1) on the surface of sutures is one of the approaches adopted to impart antimicrobial activity to sutures. The application of chitosan coating to a suture material has a benefit of smoothing suture surface, combating bacterial colonization and enhancing certain other properties (Adekogbe and Ghanem 2005).

**Figure 6.1 Molecular Structure of Chitosan**

Chitosan is the deacetylated derivative of chitin, which is the main component of the shells of crustaceans such as shrimps, crabs and lobsters. Chitosan has been found to inhibit the growth of microbes in a large body of work that has been reported by several researchers (Yuan and Robin 2008). The antimicrobial mechanism is not clear but is generally accepted that the primary amine groups provide positive charges which interact with negatively charged residues on the surface of microbes. Such interaction causes
extensive changes in the cell surface and cell permeability, leading to leakage of intracellular substances.

The antimicrobial ability, coupled with its non-toxicity, biodegradability and biocompatibility, is facilitating chitosan’s emerging applications in biomaterials engineering (Rinaudo 2006). In order to impart antimicrobial activity, chitosan is successfully applied on to various suture materials namely polylactic acid sutures (Hu et al 2009), polypropylene sutures (Saxena et al 2011), polyester sutures (Gupta et al 2010) cotton sutures (Shanmugasundaram et al 2006). Even though several attempts have been made to use chitosan polymer as antimicrobial agent, its use along with silk suture has not been explored.

Frictional properties of textile materials are normally measured over a range of applied normal loads and sliding speed. A large number of studies have been carried out on the frictional properties of textile fibre assemblies. The effect of applied normal loads and sliding speed on the frictional characteristics of friction spun yarns was investigated by Ramkumar et al (2003). In another study Das et al (2007) explored the frictional characteristics of woven suiting and shirting fabrics. Ramkumar et al (2004) studied the effect of applied normal loads and sliding speed on the frictional characteristics of needle punched non-woven fabrics. Bhuvana et al (2006) investigated the frictional properties of chitosan treated wool fabrics. In another study Jeong et al (2006) characterized the frictional properties of chitosan treated wool fabrics using the Kawabata evaluation system. From the literature it is observed that there is no published data available on the frictional properties of chitosan coated suture materials.
The tenacity and knot strength are critical for secured suturing as reported by Hristov et al (2004). Low strength sutures easily break while making a knot during surgery hence surgeon have to make a knot again. If the suture breaks after surgery, then the wound remains open and more chances for infection (Von-Fraunhofer et al 1985). The changes in the mechanical properties of chitosan treated silk sutures have not been reported in the literature.

In this chapter chitosan is applied on to the braided silk sutures at three different concentrations. The effects of chitosan treatment on the frictional, tenacity, knot strength and antimicrobial characteristics of braided silk sutures are studied. Chitosan treated silk sutures are implanted onto Wistar male rat and In vivo antimicrobial activity of the silk sutures is also studied. The properties of chitosan treated silk sutures are also compared with commercial silk sutures.

6.2 MATERIALS AND METHODS

6.2.1 Materials

*Bombyx mori* silk filaments have been used as starting materials for the study. Chitosan polymer (degree of deacetylation 0.82 viscosity 300 cps) is supplied by Otto chemicals, India. All other chemicals are analytical grade and used as received.

6.2.2 Methods

The process of manufacture of chitosan treated antimicrobial silk suture is presented in the Figure 6.2. The following section discusses the each step in detail.
Figure 6.2  Process of Manufacture of Antimicrobial Silk Suture with Chitosan
6.2.2.1 Isolation of silk fibroin

Sericin was removed by treating the raw silk filaments in an aqueous solution of 0.1% (w/v) Na$_2$CO$_3$ at 98–100°C for 30 minutes as reported by Liu et al (2007). The sericin free silk filaments were subsequently washed with copious water to remove Na$_2$CO$_3$. The silk filaments were subsequently dried and conditioned at a tropical atmosphere of 27°C and 65% relative humidity for 48 hours.

6.2.2.2 Manufacture of braided silk filaments

Circular braiding machine with 16 carrier arrangement was used to manufacture braided silk filaments. The detailed method of manufacture has been described in the chapter 3, section 3.3.2. The braided silk suture with braid angle 37.1 (Structure C) was used for the subsequent study due to its superior properties as compared with other structures.

6.2.2.3 Preparation of chitosan solution and coating on to silk filaments

Chitosan solutions of different concentrations 1%, 2% and 3% (w/v) were prepared in 2.0% (v/v) aqueous acetic acid by stirring the dispersion for 1hr at 60°C. The silk braided suture (Structure C) was then treated in chitosan solutions of different concentrations for 24 hrs at room temperature. After chitosan coating, the silk braided sutures were washed three times with 0.1 M NaOH solution and with deionized water in order to remove the contained acetic acid in the chitosan film (Hu et al 2009). The silk braided sutures were subsequently padded and dried at 100°C for 10 minutes.
6.2.2.4 Friction measurement

Lawson Hemphill Dynamic Friction Tester was used to measure the frictional properties of braided silk sutures. The detailed test procedure has been explained in the chapter 3, section 3.3.5. The treated and untreated braided silk sutures were tested for friction at a constant sliding speed 150 m/min and input tension 60 cN. For all the friction testing 180 degree wrap angle was maintained and for each set of experiment 20 tests are conducted.

6.2.2.5 Tenacity and knot strength measurement

The tenacity and knot strength of silk braided sutures were determined using Instron tensile tester. 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 as described previously. The details of the knot tying procedure have been explained in chapter 3, section 3.3.4. For each test method at least 20 readings are taken.

6.2.2.6 SEM analysis

The surface characteristics of silk filaments were studied using a JSM 6390, scanning electron microscope, after coating them with silver.

6.2.2.7 Antimicrobial activity evaluation

The chitosan treated and untreated silk filaments were evaluated for its antimicrobial activity using the Agar diffusion test SN 195920-1992 (Swiss Norm) and Shake flask method (AATCC 100). The detailed test procedure is explained in the chapter 3, section 3.3.9. All of the tests are carried out in triplicate and the results are averaged.
6.2.2.8 Suture Implantation

The studies were carried out in accordance with institutional animal ethical guidelines for the care of laboratory animals of KMCH College of Pharmacy, Coimbatore, India. The study was initiated after the approval of institutional animal ethical committee (Approval No. KMCRET/Ph.D/41/09). Chitosan treated silk suture and commercial silk sutures were implanted onto Wistar male rat as per the standard procedure explained in the chapter 3, section 3.2.11.

6.2.2.9 Histological Studies

Implanted materials were dissected, and at least 0.5 cm of surrounding tissue was excised, gently rinsed in saline, fixed in a 4% paraformaldehyde solution, dehydrated, and embedded in paraffin. Seven-micron-thick sections were obtained (Leitz Wetzlar microtome, France), stained with hemalun-eosin, and photographed using Q Capture Pro Software (Qimaging, Canada).

6.2.2.10 Statistics

The datas are expressed as mean ± standard deviation. Statistical analysis was carried out using the unpaired student’s t test. A value of P <0.05 has been considered to be statistically significant.

6.3 RESULTS AND DISCUSSIONS

6.3.1 Effect of Chitosan Coating on Friction

Braided suture structures are difficult to pass through tissue because of tissue drag and cause a greater extent of tissue damage. Also, braided sutures provide higher frictional values than the monofilament
sutures. Therefore braided sutures structures are given special surface coatings to reduce the friction. According to Zurek and Frydrych (1993), the common form of characterizing the frictional properties of yarns and filaments is the coefficient of friction.

![Figure 6.3 Effect of Chitosan Concentration on Frictional Coefficient](image)

**Figure 6.3 Effect of Chitosan Concentration on Frictional Coefficient**

The effect of chitosan coating on friction coefficient is shown in the Figure 6.3. It can be observed from the Figure 6.3, that the chitosan treated sample shows lower coefficient of friction for all the chitosan concentrations than the untreated silk sutures. This may be due to the uniform film formation of chitosan on the surface of the chitosan treated silk braided sutures. This is in agreement with the studies carried out by other researchers (Jeong et al 2006). The SEM micrographs of the 3 % chitosan treated silk sutures and untreated silk braided sutures are shown in the Figure 6.4. The photograph reveals the uniform deposition of chitosan on the surface of the treated silk braided sutures.
Figure 6.4  SEM Micrographs (a) Untreated Silk Braided Sutures
(b) 3% Chitosan Treated Silk Braided Sutures
6.3.2 Effect of Chitosan Coating on Tenacity

Surgeons prefer suture materials which possess good tensile properties. If the suture 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. Figure 6.5 shows the tenacity of chitosan coated and uncoated silk braided sutures. From the Figure 6.5 it can be observed that there is an increase in the tenacity as the chitosan concentration increases from 1 to 3%. This may be due to the better binding of fibres in the silk braided sutures by the chitosan thereby offering better resistance to the axial load. These results are in line with the previously data published by Kavitha et al (2009).

![Figure 6.5 Effect of Chitosan Concentration on Tenacity](image)

6.3.3 Effect of Chitosan Coating on Knot Strength

The effect of chitosan coating on the knot strength of silk braided sutures is presented in the Figure 6.6. From the Figure 6.6, it can be observed that for the chitosan treated silk braided sutures there is a marginal increase in
the knot strength as the chitosan concentration increases from 1 to 3% than the untreated silk braided sutures. This may be due to the fact that the chitosan treated silk braided sutures have lower coefficient of friction value than the untreated silk braided sutures therefore during knot construction the knot region is not easily weakened as untreated silk braided sutures.

![Graph showing the effect of chitosan concentration on knot strength](image)

**Figure 6.6 Effect of Chitosan Concentration on Knot Strength**

Furthermore, it is observed that, for the untreated and treated silk braided sutures the knot strength is lower than the tenacity as shown in Figure 6.5 and 6.6. These observations are consistent with other reports of the knot being the weakest part of any suture when subjected to tension (Carr et al 2009). The breakage of structure taken place at the knot rather than along the suture strand, demonstrating the knot itself causes an area of high stress concentration. Some reasons may contribute to failure happening at the knot rather than along the suture. First, breakage at the knot may be caused by forces being oriented at the knot at an acute angle to the suture axis. Secondly, the suture yarn in the knot region may be weakened during knot construction and during loading. Thirdly, tightening of the knot and the
friction between yarns in the knot may contribute to the failure (Abdessalem et al 2009).

6.3.4 Effect of Chitosan Coating on Antimicrobial 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 that most wound infections begin around material left within the wound, and that the incidence of post surgical complications are directly related to the degree of contamination at the time of material placement. Measures for reducing the risk of surgical site infections include surgical technique, appropriate antimicrobial agent, adjunctive strategies for reducing wound contamination and promoting wound healing (Harnet et al 2009).

In this study chitosan has been used as an antimicrobial agent to provide protection against various microorganisms. Chitosan has been found to inhibit the growth of microbes in a large body of work that has been reported by several researchers (Yuan and Robin 2008). Chitosan is a very attractive material because it exhibits various promising biological activities such as antimicrobial activity, biocompatibility, low toxicity, complete biodegradability hemostatic activity, scar preventability and acceleration of wound healing (Rinaudo 2006). The antimicrobial activity of both the chitosan treated and untreated silk braided sutures are assessed using Agar diffusion method and Shake flask method. The Gram-positive *S.aureus* (ATCC 6538) and the Gram-negative *E.coli* (ATCC 11230) are used as test microorganisms in this study. The reason for the same has been explained in the chapter 4, section 4.3.8.
Figure 6.7 Zone of Inhibition as a Function of Chitosan Concentration

The Zone of inhibition values for chitosan treated silk braided sutures against both *E. coli* and *S. aureus* are shown in Figure 6.7. For the untreated silk braided sutures, no zone of inhibition is found against both *E. coli* and *S. aureus*. For the treated silk braided sutures, antimicrobial activity is better against both bacteria as shown in the Figure 6.8 & 6.9. The most accepted mechanism for microbial inhibition by chitosan is the interaction of the positively charged chitosan with the negatively charged residues at the cell surface of many fungi and bacteria, which causes extensive cell surface alterations and alters cell permeability. This causes the leakage of intracellular substances such as electrolytes, UV-absorbing material, proteins, amino acids, glucose and lactate dehydrogenase. As a result, chitosan inhibits the normal metabolism of microorganisms and finally leads to the death of these cells (Sang-Hoon and Samuel 2003), *S. aureus* being a Gram-positive bacterium has a thicker cell wall and hence it is more resistant to chitosan.
than *E. coli*. This fact is well documented by the previous studies also (Giridev et al 2009).

**Figure 6.8** Antimicrobial Activity of 3% Chitosan Treated Silk Suture against *S.aureus*

**Figure 6.9** Antimicrobial activity of 3% Chitosan Treated Silk Suture against *E.coli*

The bacterial reduction % for untreated and chitosan treated silk braided sutures against *E.coli* and *S.aureus* bacteria determined by shake flask
method has been presented in the Figure 6.10. From the Figure 6.10, it is observed that the bacterial reduction % is better at higher chitosan concentration against both bacteria. Furthermore at lower concentrations, chitosan is more effective against Gram-negative bacterium than Gram-positive bacterium. This may be due to fact that *S.aureus* being a Gram-positive bacterium has a thicker cell wall and hence it is more resistant to chitosan than *E. coli* (Sang-Hoon and Samuel 2003).

![Figure 6.10 Bacterial Reductions as a Function of Chitosan Concentration](image)

**Figure 6.10** Bacterial Reductions as a Function of Chitosan Concentration

Further, it is generally agreed that the bacterial reduction rates of at least 99% are needed to retard the exponential growth of most microorganisms (Orhan et al 2007). From the Figure 6.10, it is also observed that at 3% chitosan concentration, the bacterial reduction % is 100% against both *S.aureus* and *E. coli* bacteria. Therefore this study suggests that silk sutures treated with 3% chitosan are appropriate to retard the exponential growth of *S. aureus*, a Gram-positive bacterium and *E. coli* a Gram-negative bacterium.
6.3.5 Comparison of Properties of Commercial Silk Sutures with Chitosan Treated Silk Sutures

The properties of commercial silk sutures and 3% chitosan treated silk sutures are presented in the Table 6.1. From the Table 6.1, it is noted that the tenacity and knot strength of chitosan treated silk sutures are significantly higher to that of commercial silk sutures. Also, the coefficient of friction is found to be significantly lower than the commercial silk sutures. The antimicrobial activity of chitosan treated sutures is found to be significantly higher than the commercial silk sutures against both *E.coli* and *S.aureus* bacteria.

<table>
<thead>
<tr>
<th>Property</th>
<th>Commercial silk suture</th>
<th>Chitosan treated silk suture (3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenacity (cN/tex)</td>
<td>37.1±2.3</td>
<td>39.7± 2.6</td>
</tr>
<tr>
<td>Knot strength (cN/tex)</td>
<td>26.9±1.7</td>
<td>29.6±1.9</td>
</tr>
<tr>
<td>Friction</td>
<td>0.230±0.060</td>
<td>0.210±0.045</td>
</tr>
<tr>
<td>Antimicrobial activity (Zone of inhibition)</td>
<td>No clear zone is formed against both <em>E.coli</em> and <em>S.aureus</em> bacteria</td>
<td>13 mm against <em>E.coli</em> 12 mm against <em>S.aureus</em></td>
</tr>
</tbody>
</table>

6.3.5.1 In vivo studies

Wistar male rats are used for the in vivo studies. First, the abdomen is shaved, disinfected, and then draped in a sterile fashion. A vertical incision is made on the abdominal midline, and silk sutures are implanted by
continuous suturing in abdominal intramuscular position as shown in Figure 6.11. After 12 days, commercial silk sutures and chitosan treated silk sutures are explanted and subjected to histological evaluation.

![Figure 6.11 Continuous Suturing with Chitosan Treated Silk Suture](image)

From the in vivo animal studies it is observed that, commercial silk sutures exhibit numerous bacterial colonies and significant acute inflammation as compared to chitosan treated silk sutures. The chitosan treated silk sutures shows higher tissue integration, no granuloma and infection as shown in Figure 6.12. The epidermis shows numerous cells in an extracellular matrix and scar prevention in the outer regions and negligible infection as shown in Figure 6.13. These results could be explained by chitosan properties (wound healing sequences acceleration by stimulating cell adhesion and proliferation) and could explain the widely use of chitosan as a wound dressing, cell delivery platform or tissue engineering applications (Saxena et al 2011). Due to biocompatible and biodegradable properties, the use of chitosan as excipient in drug delivery has been increased in the recent years.
Figure 6.12 Chitosan Treated Silk Suture with Surrounding Fibrosis

Figure 6.13 Wound Showing Granulation Tissue Formation with Multinucleated Giant Cell
6.4 CONCLUSIONS

- Silk suture produced at braid angle 27.1 is treated with chitosan solution at three different concentration and the effects of chitosan treatment on the suture properties are studied.

- From friction results it is noted that, there is a significant reduction in friction values for the chitosan treated silk sutures. Silk sutures treated 3 % chitosan concentration showed lowest friction value of 0.2134, whereas untreated silk sutures showed highest friction value of 0.3250.

- The tensile result showed that the tenacity and knot strength of silk braided sutures increases with the increase in chitosan concentration. Silk sutures treated with 3 % chitosan concentration showed highest tenacity and knot strength of 39.7cN/tex and 29.6cN/tex respectively. Furthermore it is observed that for all the chitosan treated silk sutures the knot strength is considerably lower than the tenacity.

- The silk braided sutures treated with higher chitosan concentration exhibits excellent antimicrobial activity against both the bacteria. At 3% chitosan concentration, a zone of inhibition of 13mm and 12mm is obtained against E.coli and S.aureus bacteria respectively. Furthermore at 3% chitosan concentration, 100 % bacterial reduction % is obtained against both the bacteria. This result suggests that silk sutures treated with 1% TCH are appropriate to retard the exponential growth of S. aureus, a Gram-positive bacterium and E. coli a Gram-negative bacterium.
The properties of commercial silk sutures and 3% chitosan treated silk sutures are studied. It is noted that the tenacity and knot strength of chitosan treated silk sutures are significantly higher than commercial silk sutures. Also, the coefficient of friction is found to significantly lower than the commercial silk sutures. The antimicrobial activity of chitosan treated sutures is found to be significantly higher than the commercial silk sutures against both *E. coli* and *S. aureus* bacteria.

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