A study of the antimicrobial efficancy of silk suture

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Abstract: In recent years many antimicrobial sutures have been developed in a quest to deal with the problem of surgical site infections in the medical world. This study was done to find out the effects of pre-treating the braided suture materials before applying the antibacterial coating agent. 0.1N sodium hydroxide (NaOH) solution was used to pre-treat / pre scour the sutures to remove impurities and also to open the structure of the silk sutures so as to allow the coating agent to penetrate into the suture structure. A biodegradable polymer and an antibacterial drug, that is, polycaprolactone (PCL) and Sulphamethoxazole trimethropm (SMZ) were used as the coating agent. The polymer PCL was made into a solution of 10% whilst the drug SMZ was made into a solution of 2500µg/ml. 5cm pieces of silk braided sutures were first cut aseptically and then washed in 0.1N NaOH solution and dried. After this pretreatment, they were coated with the antimicrobial coating agent and then dried. The pretreated sutures were then evaluated and compared, with non-treated sutures acting as the control. Antimicrobial tests were then carried out to find out the effects of pre-treated and non-pre-treated sutures on the antimicrobial properties of the sutures. Through this study it was found that the pre-treated sutures exerted a better sustained efficacy assay compared to those that are not pretreated, however mechanical properties were lowered but were still within the required standards for antimicrobial sutures.

Keywords: Silk suture, antimicrobial, scouring, tenacity, surface morphological analysis

1. INTRODUCTION

The development of infection oozing and scar at the incision site following suturing is among the main concerns that surgeons, physicians and patients have in the medical field (Farid et al., 2008). The role of suture material in the development of wound surgical site infections has been the subject of speculation among surgeons since the 1960s (Alexander et al., 1967). A numerous number of bacteria may contaminate the suture material and once tissue material becomes contaminated, wound healing may become very difficult (Rodeheaver et al., 1983; Uff et al., 1995).Therefore sutures impregnated or coated with antibacterial agents have been developed in an attempt to reduce bacterial adherence and colonization of suture materials (Liu, et al., 2010).

Silk fibers in the form of sutures have been used for centuries due to their ability to offer a wide range of properties and their use as a material for wound ligation (Altman et al., 2003). Silk fibers as a suture material are well known for their impressive mechanical properties, biocompatibility, environmental stability and morphological flexibility (Vepari & Kaplan, 2007). Any suture product of natural or synthetic composition and of mono or multi-filament construction

is susceptible to bacterial attachment and colonization.

Polycaprolactone (PCL) is a widely used polymer in the field of medical research due to its appealing properties as linear aliphatic polyester that is biodegradable whose biocomaptibilty, low melting and elastomeric properties make it even more appealing for use in tissue engineering. To date, biodegradable polymers have been successful in the development of antimicrobial sutures as shown by a considerable decrease in the bacterial adherence to triclosan-coated sutures invitro. Therefore, a continued research in the development of sutures with antimicrobial properties would be worthwhile to further lay a foundation in their development (Vila et al., 2008; Gao et al., 2011).

This research was done so as to find out the effects of pre-scouring sutures with sodium hydroxide solution on the resultant antimicrobial coated silk sutures. Suture pre-treatment with sodium hydroxide (NaOH) is therefore very important in exploring the methods of producing antimicrobial coated silk sutures which can posses the properties that match the ones of the current ones used in the medical field.

2. MATERIALS AND METHODS

2.1. Suture material

The silk suture material that was used was procured from Jiangsu Medical Supplies Ltd Co. in China. It was a size 2-0 silk suture of 0.320-0.331 mm diameter. This suture was cut into 5 cm pieces for antimicrobial testing and this was done inside a laminar air floor to create an aseptic condition.

2.2. Coating agent

The suture coating agent consisted of an antimicrobial agent mixed with a drug coating carrier. Compound Sulphamethoxazole (SMZ) tablets where purchased and used as the antimicrobial agent. A biodegradable polymer, Polycaprolactone (PCL) of 80, 000 molecular weight was chosen to act as the drug carrier or polymer add on for the suture. This was dissolved together with the drug in aqueous acetic acid. 2500 µg/ml antibacterial agent was first suspended in acetic acid and homogenized at high speed for 10 minutes to reduce the gathering of particles. Then, PCL was added into the resulting suspension and stirred for 1 hr at high speed to build up a drug concentration of 10% PCL/SMZ solution (Viju, et al., 2013).

2.3. Test organisms

To test the antimicrobial properties of the prototype sutures , two test strains where used , that is, Staphylococcus Aureus (ATCC25923) and Escherichia coli (ATCC25922) as they are the most common enteric pathogens (Ming et al., 2008). All the strains were cultured to late logarithmic growth phase on agar plates at 37˚C for 18 hrs before conducting tests under aseptic conditions in a laminar airflow (Janiga et al., 2012). The colonies were touched with a loop and then transferred to a tryptone soy broth (TSB) and incubated at 37˚C until the growth reached turbidity equal to or greater than that of 0.5 McFarland standard. The

culture was then diluted using broth to give a turbidity of 1 x 108 (CFU)/ml bacterium concentration.

2.4. Suture pretreatment

The silk suture was cut into 5cm pieces and sterilized in autoclave at 121˚C. After this, sutures were divided into two, whereby the first batch was to be coated without pre-treatment and then the other batch was scoured first before antimicrobial coating using 0.1N Sodium hydroxide solution (NaOH) and then washed in distilled water (Lou et al., 2008; Janiga et al., 2012).

2.5. Coating process

A uniform coating along the length of the suture was done through the use of a roller drying system thus coating the sutures with the drug polymer combination using a dip coating method (Masini et al., 2011).

2.6. Antibacterial activity

2.6.1. Zone of inhibition Assay

A qualitative agar diffusion test was carried out on the coated silk sutures of 5 cm in length (Elayarajah et al., 2011). It was done according to the Antimicrobial Performance Evaluation (Zone of inhibition assay) Standard (ISO 20645:2004) Textiles Evaluation of antibacterial properties - Part 1: agar diffusion method (Singh et al., 2005; Pinho et al., 2011). The antimicrobial braided silk sutures with and without pre-treatment with sodium hydroxide solution were challenged in vitro with indicator strains of the selected test organisms. The zone of inhibition diameter was done according to the Fig.1. The inhibition zone diameter was calculated according to the following formula (KimiranErdem et al., 2008): -

$$H= (D-d)/2$$

Where:

H is inhibition zone (mm);

D is the total diameter of specimen and inhibition zone (mm);

d is the total diameter of specimen (mm)

2.7. Sustained efficacy Assay

After 24 hrs of incubation at 37°C, suture samples were evaluated by zone of inhibition assays, as described above and then transferred daily onto new Petri plates growing a similar number of bacteria. This assay was terminated when the sutures ceased to inhibit bacterial growth similar (Ming et al., 2007).

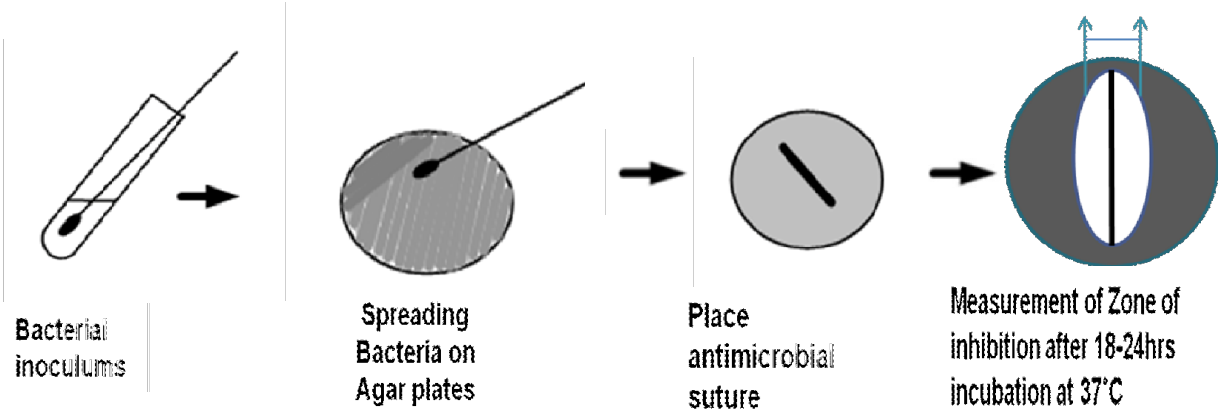

Figure 1 Measuring the Zone of inhibition

2.8. Surface morphological analysis

To observe the effect of scouring sutures before antimicrobial coating on the suture, the scanning electron microscope (SEM) was used. SEM images of sutures pre-treated with NaOH and none pre-treated sutures where obtained and then compared with SEM images of sutures without antibacterial agent.

2.9. Mechanical evaluation

2.9.1. Measurement of diameter

The diameters of the treated sutures were measured after coating and drying at five different positions along the length of the suture using an electronic thickness gauge CH-10-AT. The average diameter of each suture was calculated and expressed in millimeters. The change in diameter was calculated using the following relationship.

Change in diameter = (D1-D2) / D1

Where

D1 is the diameter of suture before coating and

D2 is the diameter of suture after coating.

3. TENSILE PROPERTIES

The tensile properties were measured using the universal testing system (YG-B026G, China). According to ASTM D2256 Standard Test Method for Tensile Properties of Yarns by the Single Strand Method, the sutures were tested for breaking tenacity, elongation at break, initial modulus and fracture strain, stress and work. The gauge length used was 50 mm and extension rate was 200 mm/min. The tests were done under a standard atmosphere for textile testing which involved a temperature of 20 ± 2 ˚C and relative humidity of 65±2% RH.

3.1. Results and discussion Antibacterial activity Zone of inhibition Assay

All the sutures pretreated by washing with NaOH (scouring) showed larger zones of inhibition diameters. This shows that the scouring treatment opens up the polymeric

braided structure of the silk sutures, therefore enlarging the diameter of the suture and at the same time allowing more antibacterial agent particles to penetrate the braided suture structure. This explains as to why a scoured suture is able to retain more antibacterial agent than non-scoured suture as illustrated by Fig. 2.

Table 1 Zone of inhibition diameter for scoured and not scoured sutures

Suture Treatment	Zone of inhibition (mm) (mm)	
	S. aureus	E. coli
Scoured	9.8±0.86	11.1±0.78
Not scoured	8.5±0.74	9.2±0.70

3.2. Sustained efficacy assay

The sutures that were scoured first by pre-treatement with 0.1N NaOH showed longer lasting antimicrobial activity as compared to the non-scoured sutures. Zones of inhibition of pre-scoured sutures where observed against both the test strains, E. Coli and SA with antimicrobial activity lasting for up to 5 days whereas the non-scoured sutures lasted only 4 days as shown in Table 2. Table 2 also shows that pre-treated sutures had larger zone diameters accounting for greater penetration of antibacterial agent into the suture braided structure.

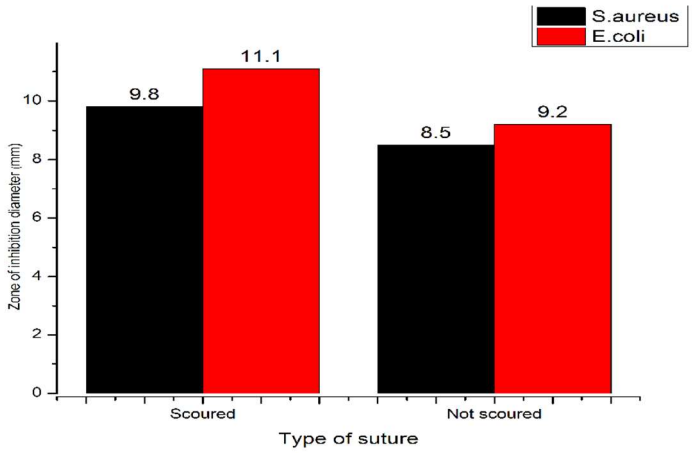

Figure 2 Zone of inhibition Assay

Table 2 Sustained efficacy assay of sutures against S.aureus and E.coli

	Zone of inhibition (mm)			
	S.aureus		E.coli	
Time (Days)	Scoured	Not scoured	Scoured	Not scoured
1	11.1±0.78	8.5±0.74	9.8±0.86	9.2±0.70
2	9.8±0.86	7.1±0.69	8.8±0.65	7.82±0.74
3	6.3±0.62	3.8±0.51	5.4±0.51	3.97±0.67
4	4.1±0.55	2.77±0.46	3.8±0.47	3.32±0.41
5	2.60±0.47	0	1.5±0.34	0
6	0	0	0	0

3.3. Surface morphological analysis

Figure 3 above shows a rougher surface on the suture pre-treated with NaOH (c) as compared to suture (b) which was not pre-treated. The SEM images show that a stable and regular coating was applied on the surface of the suture after the addition of PCL into the coating agent. This shows relatively good properties for sutures as it prevents friction during suturing process. However according to the results observed in picture (c) it is seen that the polymeric structure of the silk fibers is relatively destroyed when a suture is scoured with NaOH.

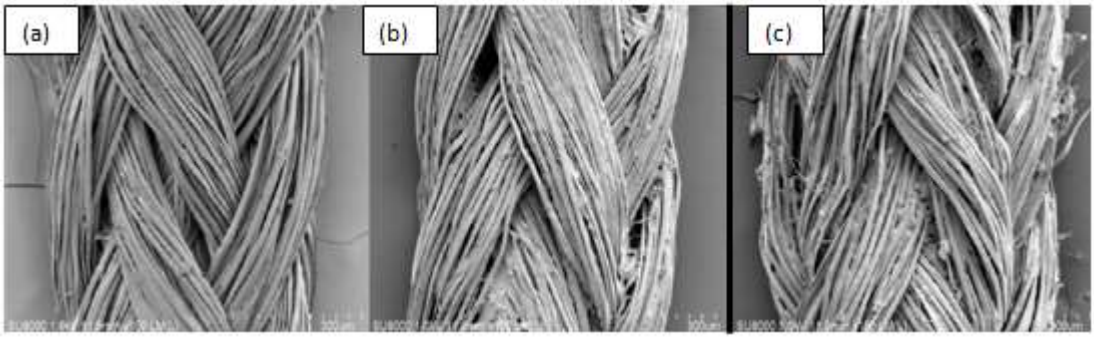

Figure 3 Scanning Electon Microscope (SEM) images showing the effects on the physical appearance of the antibacterial treated and untreated suture (a) untreated suture , (b) SMZ +PCL treated suture and (c) NaOH pretreated suture.

Therefore, as much as sutures pretreated with NaOH showed relatively good results on antimicrobial activity, its effects on the physical properties of the suture were not very good.

4. MECHANICAL EVALUATION

4.1. Measurement of diameter

The results showed that pre-treating the suture by scouring before coating the silk sutures with antimicrobial agent had a large effect on the diameter of the suture. As it is shown by the graph in Fig.4, diameter of the scoured suture is larger than that of the non-scoured suture. However, the increase observed was still within the required diameter of (0.30-0.39) experimental reference Chinese standard (YY0167-2005) for a 2-0 suture.

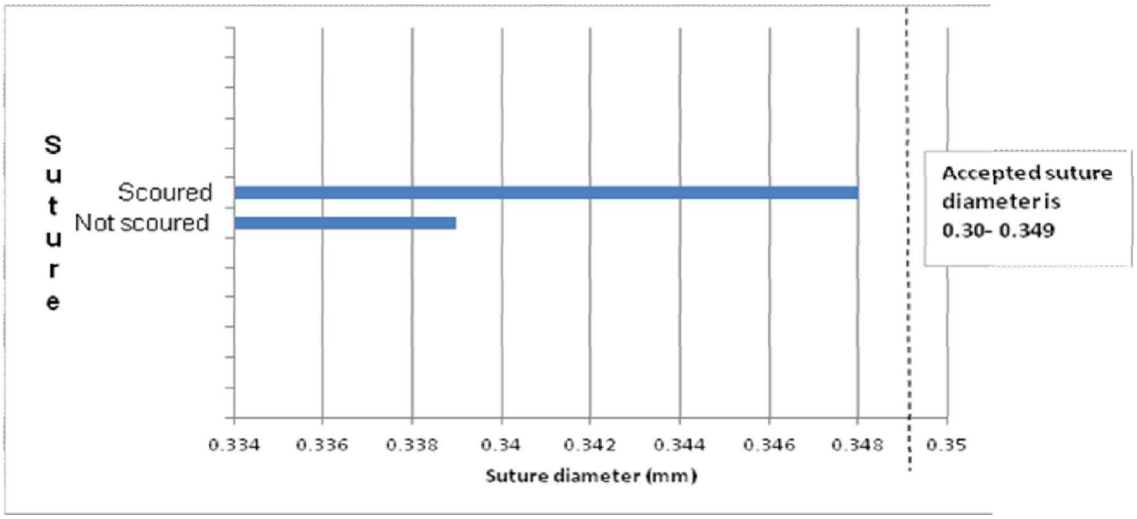

Figure 4 Effects of scouring on suture diameter

4.2. Tensile Properties

A decrease in both suture tensile properties was observed as the suture was subjected to treatment with coating agent. However more decrease was observed for sutures that had been pretreated with NaOH. The decrease in the overall strength was probably due to the suture losing some of its mechanical properties during suture pre-treatment, as shown by the surface morphological results of the suture.

Table 3 Suture Tensile properties

Property tested	Not coated	Type of suture	
		No Pre- treatment	Pre- treated with NaOH
Breaking strength (N)	27.4±1.5	24.8±1.4	20.51±2.3
Elongation at break (mm)	8.94±2.6	8.66±0.6	10.57±1.8
Initial modulus (N/mm)	94.69±2.3	92.28±0.8	90.04±0.4
Fracture stress (N/mm)	97.45±0.8	92.35±2.6	95.61±1.9
Fracture strain (%)	17.4±1.7	19.1±1.9	23.13±2.5
Fracture work (N Ÿ/mm)	100.43±1.1	104.14±2.3	104.14±2.7

5. CONCLUSIONS

A number of researchers in this field have used this method of scouring the silk sutures with NaOH first before antimicrobial treatment. This is relatively common method as observed by the author, but however through the results obtained from this study, it shows that, this can be a disadvantage when used for medical silk sutures. The antimicrobial results observed for suture pretreated with NaOH where relatively good, with all of them surpassing the non-pretreated sutures. However, when observed in the SEM, it was seen that the NaOH destroys the silk suture surface fibers as the sutures pretreated with NaOH had a rougher surface compared to those not scoured.

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