



## Interpenetrating polymer network of chitosan crosslinked silicone as medical implants

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### ABSTRACT

Medical implants the most unavoidable medical device of nowadays has many potent advantages. One of the important limitations of it in medical usage is colonization of biofilm formers on the inner surface of the implants and produce high risk for the implantable material. This study involves the evaluation of one such high rate biofilm forming bacteria, *Staphylococcus epidermidis* which prevail in the blood. The research materials silicone used for making catheters and stents was coated with chitosan and crosslinked using poly- D,L lactic acid (PDLLA) to provide anti-bacterial activity. This was achieved through a dip coating procedure. The bioefficacy against *S. epidermidis*, water absorbing ability, withstanding lysozyme degradation and biocompatibility analysis were done to understand whether the coated catheters can be more advantageous than the usual one used in hospitals normally. All the analysis showed more compatible result for the chitosan-PDLLA coated catheter samples. The bioefficacy effect of the dip coated catheters and its ability to eradicate the biofilm formation on the surface designated the modified silicone catheters as efficient implants.

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### Introduction

Adherence of bacteria, named as bacterial colonisation on medical implant surface is the first step in the outbreak of implant-related infections. Bacterial colonisation has many different growth and survival strategy. One such type of colonisation is microbial biofilm formation, which is notably a risk full factor when takes place on the surface of inserted implants. These bacteria infect the patients by producing an extensive exopolysaccharide, 'glycocalyx'. Often in combination with derivatives from the host environment, the glycocalyx form a confluent protected biofilm on tissues and implanted materials. Rapidly dividing bacteria can spread along the surface of a device within the glycocalyx of the biofilm. Some of the surface bacteria can be shed and become free to attach to a new, non-colonised surface. For deriving nutrients and to counter cationic antimicrobial agents, an ion exchange matrix is formed within the biofilm. The biofilm structure also facilitates the interaction between different bacteria. The interaction of bacteria results in co-operative activities, like cross-feeding, and promoting genetic exchange and resistance transfer. Bacteria covered by biofilm can be more resistant to antimicrobial agents even at high concentration. This makes serious complications in treatment of implant related infections<sup>[1]</sup>.

Catheter related infections are more frequent in post-operative stages of patients. Because of the size, shape and ambient environment, the biofilm formation is quite faster in implanted catheters both the outer and luminal wall of the device. A site specific active antimicrobial agent administered at high concentration can increase the success of antimicrobial chemotherapy<sup>[1]</sup>

Medical devices range from easily inserted and retrieved contact lenses, urinary catheters and endotracheal tubes to surgically implanted cardiac valves, hip joints and coronary stents<sup>[2]</sup>. Since silicone has been used successfully in many health care applications, it is tacitly understood that silicone materials are intrinsically biocompatible for preparing medical implants. Medical silicone materials, including fluids, gels, elastomers, and adhesives, are used extensively in medical field because it possess an appropriate host response at specific situations in patients. Silicone's biocompatibility in medical applications is probably related to its exceptional thermal and chemical stability properties. Silicones remain essentially unaffected by repeated sterilization by autoclaving, and they can usually be dry heat sterilized as well. Silicones can also be formulated into other biomaterials, such as polyurethane, to enhance their biocompatibility<sup>[3]</sup>.

Gaining resistance to antimicrobials is a major concern worldwide. Releasing antimicrobials at sub-inhibitory concentration to the surrounding tissues or fluids can induce resistance in infecting bacteria<sup>[4]</sup>. Both antibiotics and antiseptics are used to combat bacterial infections. However, bacterial resistance develops much less readily to antiseptics than to antibiotics<sup>[5]</sup>.

### Materials and Methods

#### Sample collection

Urinary catheters made up of silicone are the chief research material of this study. These materials are collected from the Coimbatore Government Hospital, Coimbatore, India and coated with chitosan and a drug carrier, poly DL-lactic acid (PDLLA) using dip coating procedure<sup>[6]</sup>. Medical grade chitosan was

purchased from Sigma Aldrich Co.USA and medical grade poly DL lactic acid was purchased from Hi Media Co.India.

#### Antimicrobial activity of chitosan and PDLA

Chitosan at a concentration of 0.01mg and PDLA of 1ml were used to analyse the antimicrobial activity using agar diffusion method<sup>[6]</sup>.

#### Dip-coating of silicone catheter<sup>[6]</sup>

Chitosan was used in the present study in four concentrations (0.1mg, 0.2mg, 0.3mg and 0.4mg) mixed with 4ml of poly D, L-lactic acid (PDLA) until the formation of a viscous gel. The silicone catheters (1cm x 0.5cm) were sterilized and dipcoated<sup>[1]</sup> in the viscous gel as well as in PDLA and incubated with shaking. Agar diffusion method was followed to determine the bioefficacy of chitosan and the cross-linker PDLA against the biofilm forming pathogen *Staphylococcus epidermidis*.

#### Swelling ability study of chitosan<sup>[7]</sup>

A known weight of chitosan powder was incubated in phosphate buffered saline (PBS, pH-7.4) to determine the swelling ability. The percentage water absorption of chitosan in the media was calculated as follows:

$$E_{SW} = \frac{W_e - W_o}{W_o} \times 100 \quad (1)$$

Where,  $E_{SW}$  is the percentage water adsorption of chitosan at equilibrium.  $W_e$  denotes the weight of the chitosan at equilibrium water absorption, and  $W_o$  is the initial weight of the chitosan powder. Each experiment was repeated 3 times, and the average value was taken as the percentage water absorption.

#### In vitro degradation of chitosan<sup>[7]</sup>

The *in vitro* degrading ability of the chitosan was studied using lysozyme (500-1000U/C.C.) in phosphate-buffered saline (pH7.4). The degradation percentage of chitosan was calculated as follows:

$$E_{ID} = \frac{W_o - W_f}{W_o} \times 100 \quad (2)$$

Where,  $E_{ID}$  is the percentage of chitosan degradation *in vitro*.  $W_o$  denote the initial weight of the chitosan powder and  $W_f$  denotes the final weight of the powder after lysozyme treatment. The experiment was performed in triplicate and the average value was taken as the percentage weight loss.

#### Checking the biofilm formation of pathogen using plate bioassay

Cultivation of *Staphylococcus epidermidis* in brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red (0.08%) was done to screen the biofilm formation of pathogen<sup>[8]</sup>.

Analysis of the surface adhesion and colonisation of pathogen on chitosan coated catheter as a substrate for biofilm formation was done using the biofilm plate assay<sup>[9]</sup>. Nylon membrane discs (control) and the discs of coated catheter were placed on BHI broth swabbed with 3 hours old culture of *Staphylococcus epidermidis*. After incubation, the discs were sonicated at 2 pulse for 60 seconds to detach the adhering bacteria. The dilution series of the sonicated samples were prepared and each dilution were plated and incubated overnight.

#### Tissue responses in chick chorio-allantoic membrane

Biocompatible nature of chitosan was analysed using embryonated eggs. Nine day embryonated eggs were candled to determine the position of air sac. A window was cut in the shell and silicone catheter (control) and chitosan coated catheter (1.5 x 1.5 x 0.5 mm) were placed in the chorio-allantoic membrane and sealed for incubation<sup>[10]</sup>.

#### Results

##### Antimicrobial activity of chitosan and PDLA

The antimicrobial activity of chitosan and PDLA was analysed against *Staphylococcus epidermidis* using the agar diffusion method and the result was tabulated in Table-1.

##### Dip-coating of silicone catheter

Urinary catheter pieces used for dip coating with chitosan-PDLA viscous gel was dried to get a smooth surface and for even spreading of the antimicrobial gel (Figure-1).

Figure-1: Dip-coating of silicone catheter



Figure -1 shows the picture of chitosan dip coated silicone catheter using PDLA as crosslinking agent

The agar diffusion method showed good antimicrobial activity against *S. epidermidis*, which was evident by growth inhibition zone formation (Figure-2).

Figure-2: Bioefficacy of chitosan coated silicone catheter

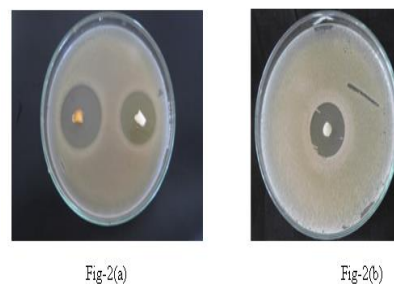


Fig-2(a)

Fig-2(b)

Figure-2(a) shows the zone of inhibition of chitosan (left) and PDLA (right) against *S. epidermidis*

Figure-2(b) shows the zone of inhibition of chitosan coated silicone catheter using PDLA as crosslinking agent against *S. epidermidis*

PDLA-chitosan coated catheter showed more bioefficacy than catheter pieces coated individually with chitosan and PDLA (Table-2).

##### Swelling ability study of chitosan

Table-3 shows the water uptake ability of the chitosan used in this study. The chitosan is a microporous structure and hence the coated silicone can hold water in each pore. The water uptake ability reaches 45.6% for silicone catheter pieces.

##### In vitro degradation of chitosan

Chitosan coated silicone catheter pieces after lysozyme degradation showed difference in weight from its initial. Table-4 shows the difference in weight before and after lysozyme degradation. The decrease in weight of catheter pieces implies the action of lysozyme on chitosan.

### Checking the biofilm formation of pathogen using plate bioassay

Formation of mild black coloured colonies in BHI-Congo red medium reveals the isolate as intermediate biofilm formers in medical implants (Figure-3). As the result confirms that the used microbial strain is a biofilm former, the organism was used further in this study to measure the imparted antimicrobial activity on silicone catheter.

**Figure-3: Checking for biofilm formation using Congo red agar plate method**



**Figure-3 shows the black coloured colonies with dry colony surface of *S. epidermidis* on Congo red agar medium, which indicates the bacteria is a biofilm former.**

### Tissue responses in chick chorio-allantoic membrane

The chorio-allantoic membrane placed chitosan-PDLLA crosslinked silicone catheter pieces were removed after incubation. The chorio-allantoic membrane was removed and stained with Eosin-Haematoxylin stain. The stained membrane was viewed at 40X in a light microscope and compared with the normal membrane.

### Discussion

*Staphylococcus epidermidis* the major bio-film forming organism and the causative agent of implant associated infections was swabbed on MH agar plates. The chitosan, PDLLA dip coated and uncoated catheter pieces were placed and incubated. The antimicrobial activity of the silicone coated chitosan was measured using the zone of inhibition formed.

The water uptake ability of chitosan was rapid and it absorbs water within 30 seconds and was saturated within 1 minute. The chitosan coated on the materials with microporous structures seem to show the difference in the water adsorption depending on the porous structure, which is attributed to the water retained within the pores.

Stability is an important preferred property for the chitosan powder during a few days of contact with the body fluids when implanted. The result represents the percentage of weight loss of chitosan coated silicone as a function of degradation by lysozyme over time. The weight loss percentage of chitosan silicone was 31.12%. Probably because of the higher cross-linking degree of PDLLA and chitosan powder it was considered stronger and so the polymerized chitosan can withstand the lysozyme degrading action<sup>[7]</sup>.

The modified method followed, namely Congo-red Agar method (CRA) was adopted to evaluate the biofilm forming ability of the catheter isolate *S. epidermidis*. The negative colonies will show red colour while the positive strains can form a dry crystalline colony surface with mild black colouration, which indicated the biofilm forming ability was moderate or intermediate. While the dark black coloured colonies forming strains are designated as high biofilm formers<sup>[11]</sup>.

The chitosan crosslinked with PDLLA loaded silicone catheter samples were implanted in the nine-day old embryonated chick eggs by placing it on the chorio-

allantoic membrane portion and incubated for 7 days. During and after the exposure period (9, 12 and 17 days), the eggs were observed for hypersensitive reactions—edema and erythema. No such reactions on the CAM surface of the chicks were observed after the specified incubation period. The stained membrane showed the normal blood vessel formation without any pleuritis symptom and hence the samples prepared in this experimentation can be considered biocompatible<sup>[10]</sup>.

### Conclusion

Medical implants, is the most vulnerable source material of surgery. After implantation it has good number of contact days with the patients in both pre- and post-operative stages. Because of the weak innate immune protection, the patients are more prone to microbial invasion. Such unwanted invasion results in the formation of biofilm in the inner surface of implants, which leads to improper functioning of the human organs with multiple infections. Chitosan, the antimicrobial agent derived from chitin of crustacean shells can be used as a coating material on implants. The cross-linker poly-DL-Lactic acid is very effective in crosslinking the chitosan on silicone catheter's inner and outer surfaces. This can be achieved using the dip coating procedure. Factors like antimicrobial action of the coated catheters, water uptake ability, in vitro degradation and biocompatibility were done to confirm its usage on patients. The results generated in this study conclude that the dip coated silicone catheters are compatible for patients and can be implanted for more contact days than the normal catheters.

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isolates of staphylococci: an evaluation of three different screening methods, *Indian Journal of Medical Microbiology*, 24 (1), 25-9, 2006.

**Table-1: Antibacterial activity of chitosan and PDLA**

Organism	Chitosan concentration (mg)	Zone of inhibition (mm)	PDLA concentration (ml)	Zone of inhibition (mm)
Staphylococcus epidermidis	0.01	15	1	9

**Table-2: Antimicrobial activity of chitosan coated silicone catheter using PDLA**

S. No	Chitosan concentration (mg)	PDLA concentration (ml)	Zone diameter (mm)
1	0.1	4	9
2	0.2	4	12
3	0.3	4	16
4	0.4	4	15
5	0.0	4	3

**Table-3: Water uptake ability of chitosan crosslinked with silicone catheter**

S.No.	Initial weight (gm)	Time of water uptake (min)	Final weight (gm)
1	0.242	5	0.412
2	0.412	10	0.420
3	0.420	15	0.433
4	0.433	20	0.445
5	0.445	25	0.445

**Table-4: In vitro degradation of chitosan crosslinked with silicone catheter**

S.No.	Initial weight of chitosan (gm)	Time of Degradation (min)	Final Weight of coated silicone (gm)
1	0.710	30	0.605
2	0.605	60	0.547
3	0.547	90	0.517
4	0.517	120	0.508
5	0.508	150	0.489

**Table-5: Checking Biofilm Formation on Chitosan coated and uncoated silicone catheters**

S.No.	Type of membrane /catheter	Number of colony forming units in diution factors			
		$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$
1	Nylon membrane(control)	TNTC	270	153	127
2	Silicone catheter	21	4	2	1
3	Chitosan coated silicone catheter	3	1	0	0