Comparative evaluation of sutures coated with triclosan and chlorhexidine for oral biofilm inhibition potential and antimicrobial activity against periodontal pathogens: An in vitro study

Kunal Sunder Sethi, Prerna Ashok Karde, Chaitanya Pradeep Joshi

ABSTRACT

Background: Surgical site plaque accumulation is one of the challenging problems leading to unfavorable healing. The antibacterial sutures can be used to reduce or inhibit plaque formation. Presently there is no study comparing efficacy of sutures coated with triclosan and chlorhexidine in terms of oral biofilm inhibition and antimicrobial property against periodontal pathogens.

Aim: The aim of present study was to evaluate the antibacterial efficacy and oral biofilm inhibition around chlorhexidine and triclosan coated polyglactin sutures in comparison to uncoated sutures.

Materials and Method: Equal segments of chlorhexidine and triclosan coated polyglactin sutures (3-0) were incubated at 37°C in saliva collected from 10 chronic periodontitis patients for 7 days. Plain uncoated suture served as control. Biofilm formation was analyzed with Confocal Laser-Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM). Quantitative assessment was done using Colony Forming Units (CFU/mL). The antibacterial efficacy of the sutures was tested against specific periodontal pathogens (S. mutans, F. nucleatum, A. actinomycetemcomitans, P. intermedia, P. gingivalis) using agar diffusion method. CLSM and SEM were not subjected to statistical analysis. ANOVA test was used for colony forming units and agar diffusion test. (P < 0.05)

Results: CLSM and SEM showed substantial biofilm inhibition around chlorhexidine-coated sutures followed by triclosan-coated when compared to plain uncoated suture. The antibacterial coated sutures showed statistically significant difference in CFUs/ml and zone of inhibition compared to plain uncoated sutures. Among coated sutures, chlorhexidine-coated sutures showed better results.

Conclusion: The antibacterial coated sutures have a promising potential in preventing the colonization of periodontal pathogens around it thereby inhibiting biofilm formation.

Key words: Chlorhexidine, periodontal pathogens, sutures, triclosan

According to the World Health Organization report, chronic periodontitis is considered to be one of the common dental diseases affecting human population with a prevalence rate of 10%–15% of the world population suffering from severe periodontitis.[1] Various clinical trials demonstrated that nonsurgical therapy is usually sufficient to resolve inflammation and arrest periodontitis. However, the treatment of persistent deep pockets using open flap debridement results in greater success.[2] Success of any surgical intervention depends on proper wound closure and absence of bacteria at the healing site. Sutures which are used for wound approximation can act as a reservoir of...
microbes at the surgical site leading to increased chances of surgical site infection (SSI).\[^{[3]}\]

Sutures used in oral cavity are continuously bathed in saliva containing 7.5 × 10^8 microorganisms/mL. This results in continuous wicking of microorganisms along the suture at the surgical site which results in a prolonged inflammatory response and SSI.\[^{[4]}\] Studies regarding the use of antibiotic–coated sutures were conducted which had mixed results in prevention of SSI.\[^{[5‑9]}\] Some studies favored its usage in prevention of bacterial load while other failed to demonstrate any added benefit. Triclosan and chlorhexidine are few examples of routinely used antibacterial agents to coat the surgical sutures. However, there is limited data regarding its application in periodontal surgical procedures. To the best of authors’ knowledge, there is no available study that has compared antibacterial efficacy of commercially available triclosan- and chlorhexidine-coated sutures against periodontal pathogens. Hence, the aim of the present in vitro study is to evaluate the antibacterial efficacy and oral biofilm inhibition of polyglactin (910) sutures coated with chlorhexidine and triclosan in comparison to uncoated sutures as a control.

**MATERIALS AND METHODS**

**Sample preparation**

Absorbable braided dyed polyglactin 910 (3-0) suture coated with Triclosan (VICRYL® Plus, Ethicon) and absorbable braided dyed polyglactin 910 (3-0) coated with Chlorhexidine (PECTRYL® CS, Dolphin suture) were cut into equal segments. Plain polyglactin 910 (3-0) suture (VICRYL®, Ethicon) served as a control for the tests. The pieces were then incubated at 37°C for 7 days in saliva obtained from ten patients diagnosed with chronic periodontitis. Patients were free of any systemic disease such as diabetes and hypertension. In addition, patients taking any medication known to affect the outcomes of periodontal therapy, smokers, immunocompromised patients, and those who had taken antibiotics in the past 3 months were also excluded from the study. All the tests except agar plate diffusion test (nonincubated sutures were used) were performed using suture segments incubated in saliva as described above.

**Tests performed**

- Using saliva incubated sutures
  - 1. Biofilm analysis
    a. Confocal laser scanning microscopy (CLSM)
    b. Scanning electron microscopy (SEM).
  - 2. Microbial analysis
    a. Total colony-forming units (CFUs/mL).
  - Nonincubated sutures
    1. Agar plate diffusion test.

**Biofilm analysis**

*Confocal laser scanning microscopy*

The biofilm on suture samples was analyzed by CLSM at National Institute for Research in Reproductive Health, Parel, Mumbai. For CLSM analysis of biofilm, sutures samples were stained with LIVE/DEAD® BacLight™ Bacterial Viability Kit (Molecular Probes) consisting of two nucleic acid dyes: Green-fluorescent SYTO9 and red-fluorescent propidium iodide. Equal volumes of each dye (3 µL) were combined in a microcentrifuge tube, and this mixture was used to stain the sutures for 1 h followed by washing with saline. Later, the samples were fixed in neutral-buffered formalin (100 µL) for 30 min. The fixed suture samples were again washed with saline and mounted on slide using mounting oil (10 µL). The samples were then examined using CLSM under 100× oil immersion objective. Excitation wavelengths of 480 nm (argon laser) and 561 nm (red DPSS laser) were used for the detection of green fluorescent SYTO9 and red-fluorescent propidium iodide, respectively. Viable bacteria exhibited green fluorescence, whereas dead bacteria exhibited red fluorescence. Series of images were captured at two different sites per suture and two-dimensional (2D) projections of the z-stacks (1 µm interval) and 3D reconstructions were obtained.

*Scanning electron microscopy*

SEM analysis was performed at EPCOS India Pvt Ltd., Nashik, Maharashtra. For SEM analysis, saliva incubated suture segments were fixed using 0.25% glutaraldehyde for 2 days and stored at 4°C. This was followed by dehydration using different ethanol volumes starting; 30%, 50%, 70%, 80%, 90%, and 100%. For each ethanol volume till 90%, incubation was done for 10 min. Final incubation in 100% ethanol was done for 1 h. The sample was then mounted on a stub and observed under 500× using SEM.

**Microbiological analysis**

*Total colony-forming units*

Suture samples incubated in saliva were agitated into test tubes containing 10 mL saline using vortex mixer. Dilution of 10^−2 was prepared, and 0.1 mL of this dilution was plated on blood agar using streak method. The agar plates were incubated aerobically at 37°C for 48 h. Colonies of bacteria were counted using classical bacterial counting technique and they were expressed as a number of CFU/mL.

**Zone of inhibition**

Agar plate diffusion tests to determine antibacterial efficacy was performed at Maratha Mandal’s NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka. The antibacterial efficacy of sutures was tested against the periodontal pathogenic strains of *Streptococcus mutans* (ATCC No: 25175), *Fusobacterium nucleatum* (ATCC No: 25586), *Aggregatibacter actinomycetemcomitans* (ATCC No: 43718), *Prevotella intermedia* (ATCC No: 25611), and *Porphyromonas gingivalis* (ATCC No: 33277). All the bacterial suspensions were prepared to 0.5 McFarland standards and inoculated on different agar plate by dipping a sterile cotton swab into the inoculums and swabbing the entire agar plate three times.\[^{[8]}\] Sutures (directly from the sealed packet cut under sterile condition) of 3 cm length were placed on these
inoculated agar plates and incubated anaerobically at 37°C for 24 h. Inhibition zones were measured (in mm), with a caliper perpendicular to the middle of the threads.

**Statistical analysis**

Statistical analysis of the results was done only for CFUs using SPSS software version 20 (Chicago, IL, USA). The ANOVA test was used for continuous variables after confirming normality of the data distribution. The method of Bartlett was used to confirm that the data had a Gaussian distribution. Statistical significance was defined as $P < 0.05$. Since SEM and CLSM were qualitative tests, they were not subjected to any statistical analysis.

**RESULTS**

**Biofilm analysis**

*Confocal laser scanning microscopy*

Stack images [Figure 1] obtained of plain and triclosan-coated suture showed the presence of red and green fluorescence. However, the green fluorescence decreased toward the interior of the suture suggesting that the bacterial viability decreased from the external surface toward the central portion of suture. Both of these sutures showed some degree of autofluorescence. Stack images of chlorhexidine showed the presence of green fluorescence toward the interior of the suture. However, when compared with plain and triclosan-coated suture, the green fluorescence was very less suggestive of bacterial inhibition potential.

3D-CLSM images [Figure 2] obtained at 100× magnification showed red and green fluorescence when plain uncoated suture was observed suggestive of abundance of bacterial adherence. Triclosan- and chlorhexidine-coated suture showed comparatively reduced presence of green fluorescence suggestive of inhibition potential of the antibacterial sutures. Chlorhexidine-coated suture had least amount of fluorescence suggestive of maximum inhibition potential.

*Scanning electron microscopy*

SEM images showed maximum biofilm formation on plain uncoated suture. The antibacterial coated sutures showed a comparatively clearer and smooth surface suggestive of minimum biofilm formation. Biofilm formation was least on chlorhexidine-coated suture [Figure 3].

**Microbiological analysis**

*Total colony-forming units*

The total CFUs [Figure 4] obtained using plain uncoated suture was highest while it was lowest with chlorhexidine-coated suture. The mean CFUs/mL for plain uncoated suture was $127 \pm 46$, whereas for triclosan- and chlorhexidine-coated suture, it was $82 \pm 41$ and $22 \pm 21$, respectively. Intergroup comparison between chlorhexidine coated and plain uncoated suture was statistically significant ($p < 0.001$). The intergroup comparison between triclosan and plain uncoated suture ($p < 0.05$) and between chlorhexidine-coated suture and triclosan-coated suture ($p < 0.01$) was significant.

Figure 1: Series of 2D CLSM images (z-stacks) of Live/Dead® (Syto-9 and PI) stained microbial biofilms on sutures obtained at intervals of 1 µm (100×) magnification [green florescence (viable bacteria)] and [red florescence (nonviable bacteria)] (a) plain suture (b) triclosan coated suture (c) chlorhexidine coated suture

Figure 2: Three-dimensional visualizations of confocal laser scanning microscopy images of Live/Dead® (Syto-9 and PI) stained microbial biofilms on sutures (100x) (→ green florescence [viable bacteria]) and (← red florescence [nonviable bacteria]) (a) plain suture (b) triclosan-coated suture (c) chlorhexidine coated suture
Gram-staining
Microscopy revealed the maximum presence of Gram-positive cocci with chains of streptococci and Gram-negative short rods associated with plain uncoated suture. Similar microscopic appearance was present with triclosan-coated suture. Chlorhexidine-coated suture, however, showed the presence of only Gram-positive cocci with the absence of Gram-negative rods suggestive of alteration in bacterial flora.

Agar plate diffusion tests
Zone of inhibition was present around both the antibacterial-coated sutures against all the tested periodontal pathogens. It was comparatively more with triclosan-coated suture [Figure 5].

DISCUSSION
In a study conducted by Katz et al. using radiolabelled bacteria, authors stated that bacterial adherence to suture materials can play a significant role in the induction of SSI.\textsuperscript{[10]} Intraoral suture removal can lead to bacteremia which is a potential risk factor for developing bacterial endocarditis in high-risk patients.\textsuperscript{[11]} Triclosan is effective in significantly reducing the bacterial adherence to suture material which can decrease the SSI and morbidity.\textsuperscript{[6]} Venema et al. in his study on sutures coated with triclosan concluded that they do not provide a sufficient antimicrobial effect to prevent \textit{in vitro} colonization by oral bacteria, whereas use in combination with a chlorhexidine-cetylpyridinium-containing antiplaque rinse appears to be counterproductive.\textsuperscript{[12]} In our study, triclosan-coated suture showed substantial reduction in biofilm formation in comparison to plain uncoated suture.

Walker et al. conducted a study using chlorhexidine (4.0%v/v) as an antibacterial coating on suture against methicillin-resistant \textit{Staphylococcus aureus} (MRSA), \textit{Staphylococcus Epidermis}, and \textit{Escherichia Coli}, with Vicryl Plus as a positive control. It was found that the chlorhexidine-coated suture and Vicryl Plus had statistically
equivalent zone of inhibition when tested against MRSA, *S. epidermis* while it was statistically more significant with chlorhexidine-coated suture against *E. coli*. In another study, chlorhexidine coatings for antimicrobial surgical sutures with three different antiseptic concentrations based on palmitic and lauric acid carriers demonstrated their high antimicrobial efficacy against *S. aureus in vitro*. The results of the present study showed a significant zone of inhibition against all the tested periodontal pathogens which were in accordance with previous studies done using chlorhexidine-coated suture.

In the present study, CLSM analysis showed maximum biofilm inhibition potential with chlorhexidine-coated suture followed by triclosan-coated suture. Similar results were obtained using SEM analysis. The CFUs were also lowest with chlorhexidine-coated suture followed by triclosan-coated suture in comparison to plain uncoated suture.

The initial colonizers of dental plaque are *Actinomyces* spp., *Streptococcus* spp., *Haemophilus* spp., *Capnocytophaga* spp., *Veillonella* spp., and *Neisseria*, while those present in mature dental plaque are *F. nucleatum*, *T. forsytsensis*, *P. gingivalis*, and *A. actinomycetemcomitans*. In the present study, antibacterial efficacy of antibacterial-coated sutures was tested against *S. mutans*, *F. nucleatum*, *A. actinomycetemcomitans*, *P. intermedia*, and *P. gingivalis* which showed a definite zone of inhibition. From the results of the present study, it is evident that both the antibacterial sutures are effective against periopathogens.

**Limitations**

Oral environment is not limited by the presence of only bacteria. It contains certain viruses, fungi, yeasts, etc. Hence, efficacy of coated sutures against this flora needs to be tested. The substantivity of the antibacterial agents coated on sutures also needs to be evaluated. This study had small sample size. Therefore, the results cannot be generalized. Further, *in vivo* studies are required to validate the results of this study.

**CONCLUSION**

Within the limitations of our study, it can be concluded that both triclosan- and chlorhexidine-coated sutures have antibacterial property against periodontal pathogens and can have a promising role in prevention of SSIs although it would require further *in vivo* validation.

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**Conflicts of interest**

There are no conflicts of interest.

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