Original Article

Comparative evaluation of the efficacy of a novel tetracycline-coated suture with triclosan-coated and noncoated sutures on bacterial load reduction: A prospective *in vitro* study

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Abstract:

Background: Surgical site infection (SSI) is one of the common postoperative complications observed after various periodontal surgeries, and sutures play a vital role in its causation. Thus, the aim of this study is to evaluate and compare the efficacy of a novel tetracycline-coated suture with triclosan-coated and nonantibacterial-coated sutures on bacterial load reduction to prevent SSI by measuring the zone of inhibition. **Materials and Methods:** Twenty systemically healthy individuals with moderate chronic periodontitis were included in this study. Fresh unstimulated saliva was collected from each patient and inoculated on three different blood agar plates. Sutures were divided into three groups (Group A: Tetracycline-coated suture, Group B: Triclosan-coated suture, Group C [control group]: Nonantibacterial-coated suture). The antibacterial efficacy of each suture was evaluated by performing agar diffusion test. The zone of inhibition around each suture was calculated, and statistical analysis was performed for the same using Kruskall–Wallis ANOVA test and Mann–Whitney U-test. **Results:** On intergroup comparison, there was a statistically highly significant difference seen for the zone of inhibition between the groups ($P < 0.01$) with the highest values in Group A (14.45 mm), followed by Group B (1.4 mm) and least in Group C (0 mm). **Conclusion:** Tetracycline-coated suture is more efficacious than triclosan-coated suture to reduce bacterial load and further prevent SSIs. However, *in vivo* clinical trial is must to prove the same.

Key words:

Agar diffusion test, surgical site infection, suture, tetracycline-coated suture, triclosan-coated suture, zone of inhibition

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INTRODUCTION

Surgical site infections (SSIs) are the second-most common postoperative complications next to urinary tract infections.[1] The most common pathogen causing these infections is *Staphylococcus aureus*, a Gram-positive bacterium, which is responsible in 23% of the cases.[2] According to Powell *et al*., of the 1,053 periodontal procedures performed, there were 22 infections, for an overall prevalence of 2.09%.[3] Surgical sutures are sterile filaments used to close wounds and provide support during the healing process.[4] However, these sutures are continuously bathed in saliva when used for periodontal surgical procedures and become the reservoir of salivary bacteria. There are few novel sutures developed to combat the postoperative infections, which include antibacterial sutures, drug-eluting sutures, stem cell-seeded sutures, and smart sutures.[5] Drug-eluting sutures can be a preferable alternative to conventional sutures as they prevent wound infections to spread and give better-wound healing.[6] Triclosan-coated suture is the first drug-eluting suture approved by the Food and Drug Administration in 2002.[5] Since then, the

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use of this suture has been increased in various general as well as periodontal surgical procedures. The most recent randomized controlled trial done by Karde *et al*. in 2019,[7] concluded that triclosan-coated as well chlorhexidine-coated sutures provide a better reduction in bacterial load and wound healing compared to conventional sutures when used in periodontal flap surgery. However, triclosan has its own disadvantages such as cross-resistance with other antibiotics, target-specific action against *Escherichia coli,* and wound breakdown.[8]

This effectuated more curiosity among the researchers, and various attempts had been done to develop a novel suture which could fight against a variety of salivary microorganisms. One such tetracycline-coated suture, developed by Shanmugasundaram *et al*. in 2011,[9] was tested against *Staphylococcus aureus* and proteus and has proved to be efficacious in reducing the bacterial load. Since tetracycline is a broad-spectrum antibiotic,[10] it can provide a better antibacterial property against salivary microflora than triclosan which has target specific activity against few microorganisms. Thus, the primary objective of this study is to evaluate and compare the efficacy of tetracycline-coated with triclosan-coated and nonantibacterial-coated sutures on bacterial load reduction to prevent SSI by measuring zone of inhibition, while the secondary objective is to evaluate the efficacy of tetracycline-coated, triclosan-coated, and nonantibacterial-coated sutures individually on bacterial load reduction to prevent SSI. The null hypothesis of this research study is "There is no difference in the efficacy of tetracycline-coated, triclosan–coated, and nonantibacterial-coated sutures on bacterial load reduction to prevent surgical site infection," while the alternate hypothesis being "Tetracycline-coated sutures have greater efficacy compared to triclosan-coated and nonantibacterial-coated sutures on bacterial load reduction to prevent surgical site infection."

MATERIALS AND METHODS

This is a prospective *in vitro* study approved by the institutional ethics committee. The sample size calculation was done using the following formula by fixing an α error of 5%, β error of 20%, and statistical power at 80%.[7]

$$n = \frac{2\,(Z\alpha + Z\beta)^2\,[s]^2}{d^2}$$

Where Zα is the z variate of alpha error which is a constant with value 1.96, Zβ is the z variate of beta error which is a constant with value 0.84 and s is pooled standard deviation which is taken as 170. According to this, the minimum sample size required in each group was calculated as 20.

A total of 20 patients who reported to the department of periodontology of our college were screened for the eligibility. A written consent was taken from all the included participants. Twenty patients (11 males and 9 females) who met the following inclusion criteria were selected. A written consent was taken from all the included participants. Inclusion criteria were age between 25–60 years and free of any systemic diseases. Patients with moderate periodontitis having 2 or more interproximal sites with clinical attachment level (CAL) ≥4 mm (not on the same tooth) or 2 or more interproximal sites with probing pocket depth (PPD) ≥5 mm, were included in the study. Exclusion criteria were smokers, immunocompromised patients, pregnant and/or lactating women, and patients who have taken antibiotics in any form in the past 3 months were excluded from the study.

Three varieties of sutures were included in this study, which were grouped as follows,

1. Group A: Tetracycline-coated sutures (4-0) braided
2. Group B: Triclosan-coated sutures (4-0) braided (Vicryl plus suture)
3. Group C (Control group): Nonantibacterial-coated sutures (4-0) braided (Vicryl suture).

Figure 1 shows a flow chart to depict a design of this study. Tetracycline-coated sutures were prepared by following the procedure given by Shanmugasundaram *et al*.[9] Figure 2 depicts the procedure performed for the preparation of tetracycline-coated sutures. Undyed Vicryl suture (4-0) was dipped into 1% sodium hydroxide for half an hour, followed by washing with distilled water. This process is called as scouring of the suture which was performed to remove natural and added impurities present in suture, in order to improve the absorbency [Figure 2b and c].

Chitosan solution was prepared by stirring a dispersion of 8 g chitosan in 2% (v/v) aqueous acetic acid solution at 60°C for 1 h [Figure 2d and e]. Then, 2 g of sodium alginate polymer was added to the chitosan solution and stirred for 10 min. The scoured suture material was immersed with this solution for about 2 h [Figure 2f]. The material was then dried at 80°C for 5 min. After drying, it was cut into 20 pieces, each of length of 3 cm [Figure 2g]. Tetracycline solution was prepared by mixing 250 mg of tetracycline hydrochloride powder in 10 ml of distilled water. Then, polymer-coated pieces of suture were dipped into this solution for 24 h followed by drying for 48 h at room temperature [Figure 2h and i].

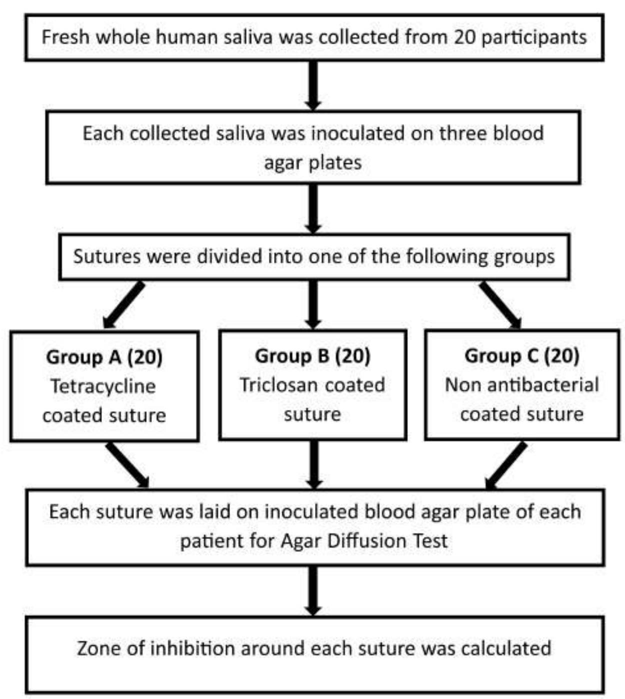

Figure 1: Flow chart of study design. *n* – number of samples

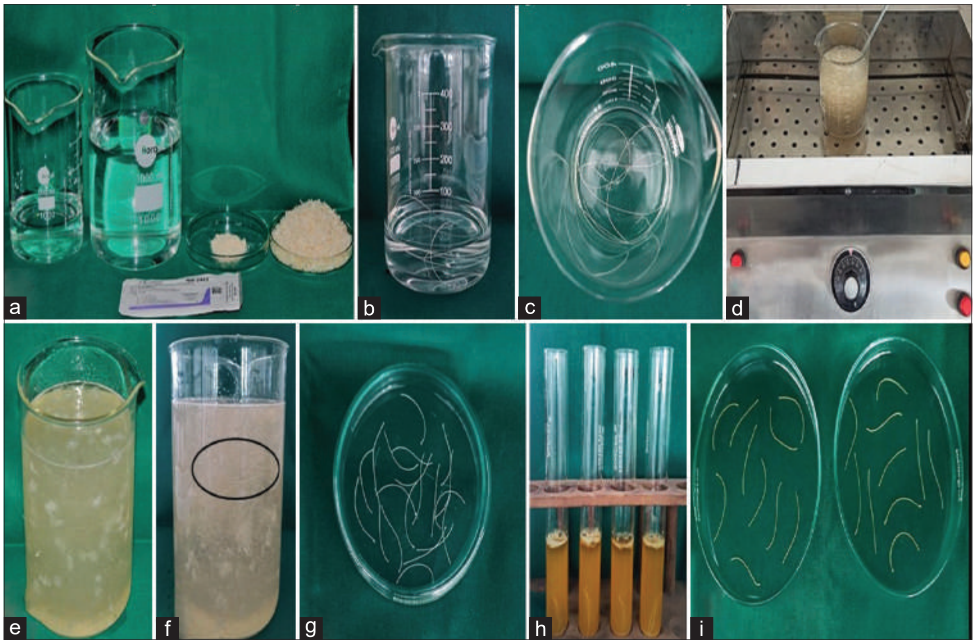

Figure 2: Procedure for preparation of tetracycline-coated sutures. (a) Armamentarium showing from left to right 1% sodium hydroxide, 2% aqueous acetic acid, 2 g sodium alginate polymer, 8 g chitosan and, undyed vicryl suture; (b and c) Scouring of suture; (d and e) Preparation of chitosan solution; (f) Placement of suture into the chitosan solution; (g) Twenty pieces of suture of 3 cm length; (h) Placement of suture threads into tetracycline solution; (i) Tetracycline-coated sutures

0.5–1 ml of unstimulated saliva was collected from 20 participants in plastic tube by suctioning using syringes. The collected saliva was immediately transferred to plastic Eppendorf tubes. Under aseptic conditions, 0.1 ml of the saliva sample was added to 0.9 ml diluents. After thorough mixing, 0.1 ml of the mixture (10^{-1}) was added to a tube containing 0.9 ml diluent and mixed again. Using this method, 10^{-6} dilution was prepared for each sample of saliva. Then, 50 µl of sample was dropped onto the surface of the blood agar plate using a micropipette, followed by placing the plates into the dry incubator at 37°C for 24 h.

The assessment of the microbiological parameters was done using the agar diffusion test. Three types of sutures were laid over the incubated agar plates. Afterward, the plates were again incubated for 24 h at 37°C, and thereafter, the growth of bacteria was determined below each suture (zone of inhibition). The presence of antimicrobial activity is indicated by the absence of bacterial growth directly below the test sample.[11]

The zone of inhibition of these sutures was calculated using the formula, H = (D-d)/2

Where H = zone of inhibition (in mm)

D = the total diameter of suture along with zone of inhibition (in mm)

d = the diameter of suture (in mm)

All data were entered into a computer by giving coding system, proofed for entry errors. Data obtained were compiled in an MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States). Data were subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). Descriptive statistics such as mean, standard deviation, and median for numerical data was depicted. The normality of numerical data was checked using Shapiro–Wilk test and was found that the data did not follow a normal curve; hence, nonparametric tests have been used for comparisons. Intergroup comparison (three groups) of zone of inhibition was made using Kruskal–Wallis ANOVA followed by pair-wise comparison using Mann–Whitney U test. For all the statistical tests, $P < 0.05$ was considered to be statistically significant, and $P < 0.01$ was considered to be statistically highly significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

RESULTS

Zone of inhibition around every suture against salivary microflora was measured in this study [Figure 3]. The zone of inhibition of Group A is 14.45 ± 0.826 mm, Group B is 1.40 ± 0.503 mm, and Group C is 0 mm [Figure 4 and Table 1]. On intergroup comparison, there was a statistically highly significant difference seen for the zone of inhibition between all groups with $P = 0$ ($P < 0.01$), having a higher value for Group A. On pairwise comparisons between three groups, there was a statistically highly significant difference seen for the zone of inhibition between Group A versus Group B, Group A versus Group C, and Group B versus Group C with $P = 0$ ($P < 0.01$) [Table 2].

DISCUSSION

SSI can hamper wound healing after periodontal surgery. Surgical sutures play a very critical role in the accumulation of saliva and thus salivary bacteria at the wound site due to their wicking action.[12] Postoperative management of patients undergoing periodontal surgery includes the administration

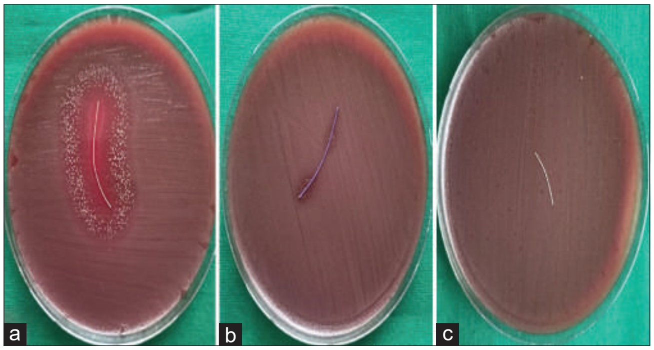

Figure 3: Zone of inhibition around sutures. (a) Zone of inhibition around tetracycline-coated suture; (b) Zone of inhibition around triclosan-coated suture; (c) Zone of inhibition around nonantibacterial-coated suture)

Table 1: Intergroup comparison of bacterial load reduction

Group	*n*	Mean±SD	Median	χ^2	*P* of Kruskal–Wallis test
A	20	14.45±0.826	15	55.389	0.000
B	20	1.40±0.503	1		
C	20	0.00±0.000	0		

SD – Standard deviation; *n* – number of samples; *P* – probability value; χ^2 – Pearson Chi-square test

Table 2: Pairwise comparison using Mann–Whitney *U*-test

Group	Versus group	Mann–Whitney *U* value	*Z*	*P* of Mann–Whitney *U*-test
A	B	0.000	–5.562	0.000
A	C	0.000	–5.847	0.000
B	C	0.000	–5.901	0.000

P – probability value; *Z* – statistical score

of systemic antibiotics, but due to lack of sustained drug delivery at the wound site, SSIs are seen after various periodontal surgeries. Thus, drug-eluting sutures were invented, and triclosan-coated suture is one of the most widely used antibacterial-coated suture. However, due to various drawbacks of triclosan, a need has arisen to find an alternative to it and tetracycline-coated suture can be a glimpse of hope. Hence, the present study evaluated if tetracycline-coated suture can be used as an alternative to triclosan-coated suture in reducing the bacterial load after periodontal surgeries.

In the present study, both the sutures, triclosan-coated and tetracycline-coated sutures showed zone of inhibition around them but a statistically highly significant difference was seen in the zone of inhibition between tetracycline- and triclosan-coated sutures, with a higher value for tetracycline-coated suture. This indicates a better antibacterial efficacy of a novel tetracycline-coated suture. It can be contributed to the broad-spectrum activity of tetracycline against Gram-positive as well as Gram-negative organisms present in saliva.[13]

Tetracycline has been used in various forms inside the periodontal pocket to treat periodontal disease. It also has anti-collagenase property which is not related to its antibacterial property.[13] Thus, proving it to be one notch higher than triclosan, which has multiple drawbacks. A randomized controlled trial by Gupta *et al*. in 2017[14] compared the plain

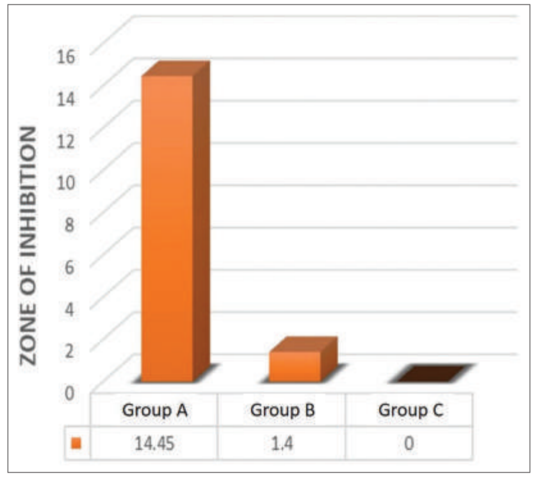

Figure 4: Intergroup comparison of zone of inhibition

sutures with sutures coated with tetracycline pomade and chlorhexidine pomade and concluded that the pomade coated sutures were effective as compared to control in reducing bacterial colonization. Similar was proved in an *in vitro* trial done by Shanmugasundaram *et al*.[9] and Viju *et al*. (2013).[15] Chitosan used in the polymer coating solution also added some advantages to the tetracycline-coated suture. It has properties of hemostasis, wound healing, and bone repair. It is also known to have anti-inflammatory and antimicrobial actions.[16] All these findings were similar to what we have achieved in this present study.

Various systematic reviews by Daoud *et al*.,[17] Edmiston *et al*. (2014),[18] Wang *et al*.,[19] Sajid *et al*.,[20] Chang *et al*.[21] have been done to evaluate the effectiveness of triclosan-coated suture and concluded that it has better antibacterial property than conventional sutures to combat the risk of SSI. The present study showed similar results in accordance with these systematic reviews. However, to the best of our knowledge, no clinical trial either *in vitro* or *in vivo* has been performed to compare the efficacy of triclosan-coated sutures versus tetracycline-coated sutures to date.

There are a few limitations of this study like *in vivo* clinical trial was not performed to evaluate clinical parameters like wound healing, also the physical characteristics of a novel tetracycline-coated suture such as tensional strength, knot strength, and drug release rate, were not measured. Future research is required in this field using different agents and antimicrobials which can efficiently protect the surgical site with minimal adverse effects.

CONCLUSION

The present study concluded that tetracycline-coated suture and triclosan-coated suture have antibacterial efficacy against salivary microflora but tetracycline-coated suture can be an alternative to triclosan-coated suture to reduce biofilm formation at the surgical site after periodontal surgeries, since it is proved to be having more efficiency against salivary microflora compared to triclosan-coated suture. However, *in vivo* clinical trials should be done to evaluate its physical as well as antibacterial properties.

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Conflicts of interest

There are no conflicts of interest.

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