Comparative evaluation of two antibacterial-coated resorbable sutures versus noncoated resorbable sutures in periodontal flap surgery: A clinico-microbiological study

Prerna Ashok Karde, Kunal Sunder Sethi, Swapna Arunkumar Mahale, Alefiya Shabbir Mamajiwala, Aishwarya Madhukar Kale, Chaitanya Pradeep Joshi[1]

Department of Periodontics, MGV's KBH Dental College and Hospital, Nashik 422 003, Maharashtra, India, [1]Institute of Dentistry, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, AB25 2ZR, Scotland

Abstract:

Background: Sutures at the surgical site can act as a reservoir for microbes, leading to surgical site infection. This mainly occurs in braided sutures due to wicking action. The use of triclosan-coated suture (TCS) or chlorhexidine-coated suture (CCS) could be one of the possible alternatives to reduce the microbial load. **Objectives:** The study was designed to assess the antibacterial efficacy of resorbable TCS and CCS along with its effect on healing after periodontal flap surgery in comparison to noncoated sutures (NCSs). **Materials and Methods:** Thirty patients with chronic periodontitis indicated for periodontal flap surgery satisfying inclusion criteria were randomly assigned in the three groups: (1) NCS-polyglycolic acid sutures (control group), (2) TCS-polyglycolic acid sutures (experimental Group A), and (3) CCS-polyglycolic acid sutures (experimental Group B). All the patients were evaluated at day 0 (baseline), day 8, day 15, and day 30 for healing index (HI), postoperative pain (POP), and visible plaque index (VPI). Aerobic and anaerobic bacterial growth around each suture was evaluated after day 8. Two randomly chosen samples from each group were examined using confocal laser scanning microscopy (CLSM) for the presence of biofilm. **Results:** Although intergroup HI and POP were statistically insignificant ($P > 0.05$), intragroup evaluation showed statistically significant improvement. VPI was more in NCS compared to antibacterial sutures. There was significantly less concentration of anaerobic bacteria as compared to aerobic bacteria ($P < 0.05$). CLSM showed the presence of more viable bacteria on NCS as compared to antibacterial sutures. **Conclusion:** TCS or CCS sutures can be used in periodontal surgeries to reduce the bacterial load at the surgical sites.

Key words:
Antibacterial sutures, chlorhexidine-coated sutures, chronic periodontitis, flap surgery, triclosan-coated sutures

INTRODUCTION

The success of periodontal surgeries is dependent on primary wound closure and absence of bacteria at the healing sites. Sutures are used for flap margin approximation and are left at the surgical sites for at least 5–7 days. However, suture surfaces, especially braided ones, have shown to provide a conducive environment for the growth of microbes at the surgical site. A long-term microbial exposure leads to increased chances of surgical site infections (SSIs) and tissue necrosis.[1] The sutures coated with antibacterial agents such as triclosan or chlorhexidine have potential to prevent the growth of these potential pathogens. Triclosan is a broad-spectrum antibacterial agent marketed for the use in oral products. It has also been demonstrated to possess anti-inflammatory property.[2] Chlorhexidine, a synthetic antimicrobial drug, is bacteriostatic at low concentration and bactericidal at higher concentration.

Thus, the use of triclosan-coated suture (TCS) or chlorhexidine-coated suture (CCS) can be a possible alternative to conventional noncoated sutures (NCSs) in preventing or reducing the incidence of SSIs. However, there is a paucity of data on the use of these antibacterial-coated

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Address for correspondence:
Dr. Prerna Ashok Karde,
Bldg No: B-2, Flat
No. 208, Akal CHS Pvt
Ltd., J. B. Nagar, Andheri
East, Mumbai - 400 059,
Maharashtra, India.
E-mail: prerna.karde@gmail.com

sutures in periodontal flap surgeries. Lack of evidence makes it difficult to reach any definitive conclusion. Hence, the present study aims to evaluate the efficacy of TCS and CCS resorbable polyglycolic acid suture after periodontal flap procedure in comparison to noncoated polyglycolic acid resorbable sutures.

MATERIALS AND METHODS

The present randomized controlled double-blinded study was approved by the institutional ethics committee and was registered with the Clinical Trials Registry – India with registration number CTRI/2016/11/007441. All clinical procedures were carried out in accordance with the Declaration of Helsinki.

Sample size calculation
The sample size calculation was done to evaluate the difference in the colony-forming units between the three groups by fixing an α error of 0.05 and statistical power at 80%. According to this, the minimum sample size required in each group was calculated as 15.

Participants
A total of 45 patients who reported to the department of periodontology of our college between May 2016 and November 2017 were screened for the eligibility. Thirty patients (mean age: 39.2 + 10.76 years; 13 males and 17 females) who met the following inclusion criteria were selected [Figure 1]. Inclusion criteria were age 25–60 years and free of any systemic diseases such as diabetes and hypertension. Only those patients indicated for periodontal flap surgery (>5 mm residual probing depth after Phase I therapy) were included in the study. Exclusion criteria were patients taking any medication known to affect the outcomes of periodontal therapy, smokers, immunocompromised patients, pregnant and/or lactating women, with any known allergies to chlorhexidine or triclosan, and who have taken antibiotics in any form in the past 3 months were excluded from the study.

Phase I therapy was performed for all recruited patients. One month following Phase I therapy, a periodontal reevaluation was performed, and patients in whom pocket depth of >5 mm persisted were selected for the study.[3,4] Thirty patients satisfied this criterion and were indicated for the periodontal flap surgery to reduce persistent periodontal pockets. One quadrant with deepest periodontal pockets was chosen in all the patients. They were then randomly divided into one of the following groups:
1. Experimental Group A: TCS-polyglycolic acid sutures. Megasorb T + ™ MERIL triclosan-coated polyglycolic suture (3–0) braided (10 patients)
2. Experimental Group B: CCS-polyglycolic acid sutures. Megasorb Plus™ MERIL chlorhexidine-coated polyglycolic suture (3–0) braided (10 patients)
3. Control group: NCS-polyglycolic acid sutures. Megasorb™ MERIL plain resorbable polyglycolic suture (3–0) braided (10 patients).

Study procedure
Following a double-blinded protocol, suture material was allocated. All suture material packets were initially placed in the opaque envelopes by one examiner (KS). One envelope was chosen randomly at the time of surgery. Conventional access flap surgery was performed in all study patients by another examiner (PK). The flaps were sutured interdentally using direct loop sutures with one of the suture materials. Suture removal was done on day 8 and analyzed for the presence of selected microbes as well as biofilm formation. Postoperative instructions were given and analgesics (ibuprofen 400 mg, TDS × 5 days) were prescribed. Antibiotics were not prescribed to any of the study patients to determine the effect of the antibacterial coating present on the experimental sutures. Warm water rinse instead of antimicrobial mouthwash was instructed twice daily for 1 min, for 30 days to eliminate confounding effect imparted by it. All the patients were recalled on day 8, day 15, and day 30.
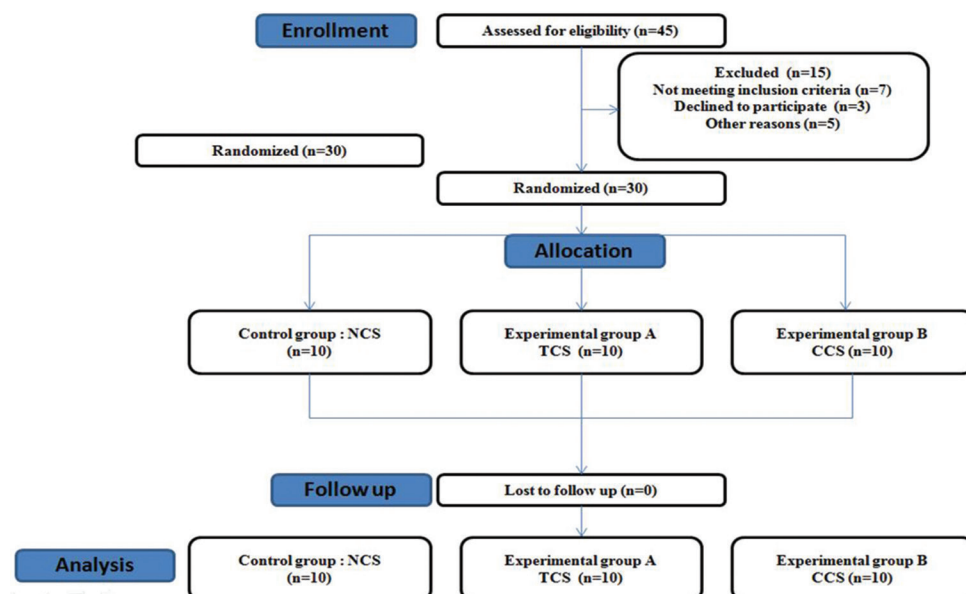
Figure 1: Consort flowchart. *n* – no of patients, TCS – Triclosan coated sutures, CCS – Chlorhexidine coated sutures, NCS – Non coated sutures

Parameters evaluated

Healing index (HI) by Landry *et al*.[5] signifies the healing after periodontal flap surgical procedure. According to this index, the scoring criteria are as follows:

1. Very poor tissue color: ≥50% of gingiva red, response to palpation: bleeding, granulation tissue: present, incision margin: not epithelialized, with loss of epithelium beyond incision margin, suppuration present
2. Poor tissue color: ≥50% of gingiva red, response to palpation: bleeding, granulation tissue: present, incision margin: not epithelialized, with connective tissue exposed
3. Good tissue color: <25% of gingiva red, response to palpation: no bleeding, granulation tissue: none, incision margin: no connective tissue exposed
4. Very good tissue color: <25% of gingiva red, response to palpation: no bleeding, granulation tissue: none, incision margin: no connective tissue exposed
5. Excellent tissue color: all tissues pink, response to palpation: no bleeding, granulation tissue: none, incision margin: no connective tissue exposed.

Postoperative pain (POP) was assessed using the numeric rating scale by Mccaffery and Beebe *et al*. (1989). In this rating, patients were asked to rate his/her pain from 0 to 10.

Visible plaque index by Ainamo and Bay[6] is one of the most commonly and reliable methods of assessing plaque. The scoring criteria is easy to interpret (i.e., visible plaque is either present or absent), so repeatability is better.

Microbiological assessment
Colony-forming units

After suture removal on day 8, it was immediately transferred into thioglycolate carrier medium and transported to the department of microbiology of our college for further analysis. A serial dilution method was performed to attain $1:10^6$ dilution. From this, 0.1 ml was transferred to six different blood agar plates (HiMedia Laboratories Pvt Ltd., Mumbai, Maharashtra, India) and evenly plated. Three of these plates were incubated aerobically at 37°C and the remaining three anaerobically at 37°C for 72 h. After 72 h of incubation incubation, the plates were removed from the incubator and colony forming units were counted. Colony counting was done for each plate and number of colonies/ml was calculated using formula: $c = n/(s \times d)$ (where c=cfu/ml, *n*=number of colonies, d=dilution factor, s=volume transferred to plate). The number of colonies/ml was calculated as the mean to obtain the total bacterial count. Colonies were examined for their morphological characteristics evaluated using a light microscope under oil immersion (×100) after Gram staining.

Confocal laser scanning microscopy

For confocal laser scanning microscopy (CLSM) analysis of biofilm, two suture samples from each group were randomly selected and stained with LIVE/DEAD® BacLight™ Bacterial Viability Kit (molecular probes). The samples were examined under ×100 oil immersion. Series of images were captured at two different sites per suture and two-dimensional projections of the z-stacks (1 μm interval).[7]

Statistical analysis

The intergroup analysis of HI and POP was assessed by Kruskal–Wallis test. The Kruskal–Wallis test (sometimes also called the "one-way analysis of variance [ANOVA] on ranks") is a rank-based nonparametric test that can be used to determine if there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable. It is considered as the nonparametric alternative to the one-way ANOVA. The intragroup analysis was performed using the Friedman test. The Friedman test is the nonparametric alternative to the one-way ANOVA with repeated measures. It is used to test for differences between groups when the dependent variable being measured is ordinal. The presence of plaque on sutures was assessed by Chi-square test, a test designed to analyze the categorical data. The intergroup analysis of CFU/ml was assessed by the one-way ANOVA test. The one-way ANOVA is used to determine whether there are any statistically significant differences between the means of three or more independent (unrelated) groups. Dunn–Bonferroni *post hoc* test was used for pairwise comparison between the two groups. The intragroup analysis of CFU/ml was performed using Student's paired *t*-test. Paired *t*-test compares the means between two related groups on the same continuous, dependent variable. For all tests, $P < 0.05$ was considered statistically significant. Statistical analysis was performed using "SPSS 9" software (IBM India Pvt LTD., Bengaluru, Karnataka, India).

RESULTS

Healing index

Intergroup comparison revealed that the distribution of HI at different follow-up intervals was same across all three categories of groups and not statistically significant. In all suture groups, intragroup comparison showed a statistically significant ($P < 0.05$) increase in the mean ranks of HI from day 0 to day 30 during the follow-up period [Tables 1 and 2].

Postoperative pain

Intergroup comparison revealed a statistically significant difference ($P < 0.05$) between the mean ranks for POP across all three groups at baseline (day 0), but there was no statistically significant difference ($P > 0.05$) between the mean ranks for POP across three groups for follow-ups. For all suture groups, intragroup comparison revealed that there was a statistically significant ($P < 0.05$) decrease in POP from day 0 to day 30 of follow-up [Tables 1 and 2].

Visible plaque index

Twenty patients showed the presence plaque on sutures on the day of suture removal, of which ten patients belonged to NCS group and five each belonged to TCS and CCS. This difference was statistically significant ($P < 0.05$).

Microbial assessment
Colony-forming units

The concentration (expressed as $\times 10^7$ CFU/ml) of aerobic as well as anaerobic bacteria was significantly lower in TCS followed by CCS, whereas it was highest in NCS ($P < 0.05$). There was significantly less concentration of anaerobic bacteria as compared to aerobic bacteria ($P < 0.05$) [Table 3]. Pairwise comparison between suture groups revealed that TCS was better than CCS and NCS for reducing aerobic ($P = 0.027$; $P = 0.001$) as well as anaerobic ($P = 0.019$; $P = 0.000$) bacterial concentration. However, CCS and NCS were comparable

Table 1: Intergroup comparison of wound healing index and postoperative pain among the three groups at different intervals

Interval	Groups	*P*	
		Wound HI	POP
Day 0 (baseline)	NCS	0.91 (NS)	0.008 (S)
	TCS		
	CCS		
Day 8	NCS	0.86 (S)	0.17 (NS)
	TCS		
	CCS		
Day 15	NCS	0.68 (NS)	0.14 (NS)
	TCS		
	CCS		
Day 30	NCS	0.98 (NS)	1 (NS)
	TCS		
	CCS		

P value obtained from Kruskal–Wallis test, Significant at *P*<0.05.
NCS – Noncoated sutures; TCS – Triclosan-coated suture;
CCS – Chlorhexidine-coated suture; NS – Not significant; S – Significant;
HI – Healing index; POP – Postoperative pain; *P* – Probability value

Table 2: Intragroup comparison of wound healing index and postoperative pain among the three groups at different intervals

Groups	Intervals	*n*	Wound HI		POP	
			Mean rank	*P*	Mean rank	*P*
NCS	Day 0 (baseline)	10	1.30	<0.05 (S)	4.95	<0.05 (S)
	Day 8		1.90		3.05	
	Day 15		3.25		1.70	
	Day 30		3.55		1.35	
TCS	Day 0 (baseline)	10	1.35	<0.05 (S)	5	<0.05 (S)
	Day 8		1.85		2.80	
	Day 15		3.05		1.65	
	Day 30		3.75		1.55	
CCS	Day 0 (baseline)	10	1.40	<0.05 (S)	4.45	<0.05 (S)
	Day 8		1.90		2.90	
	Day 15		3.05		1.65	
	Day 30		3.65		1.65	

P value obtained from the Friedman test, significant at *P*<0.05.
NCS – Noncoated sutures; TCS – Triclosan-coated suture;
CCS – Chlorhexidine-coated suture; *n* – Sample size; S – Significant;
HI – Healing index; POP – Postoperative pain; *P* – Probability value

Table 3: Colony-forming units among the three groups

Incubation	Groups	Mean±SD	*P*
Aerobic	NCS	726.5±184.74	<0.05 (S)
	TCS	444.1±104.54	
	CCS	628.4±138.47	
Anaerobic	NCS	596.3±155.64	<0.05 (S)
	TCS	356.5±81.39	
	CCS	514.6±108.94	

P value obtained from one-way ANOVA test, Significant at *P*<0.05.
NCS – Noncoated sutures; TCS – Triclosan-coated suture;
CCS – Chlorhexidine-coated suture; SD – Standard deviation;
ANOVA – Analysis of variance; S – Significant; *P* – Probability value

for aerobic ($P = 0.436$) and anaerobic ($P = 0.412$) bacterial concentration.

Confocal laser scanning microscopy
The presence of biofilm was detected on all the three types of sutures. Green and red florescence, indicating live and dead bacteria, respectively, was noted to be highest in NCS group. The florescence was noted on outer layer as well as the inner braids of NCS. The amount of florescence was reduced in both antibacterial sutures. TCS showed least amount of green florescence among all the three groups [Figures 2-4].

DISCUSSION

Postoperative complications in periodontal surgical therapy are reported to occur in 5.5% of the cases,[8] whereas another study stated a prevalence of such complications to be 2.09%.[9] SSI is one of the leading causes of under the umbrella of postoperative complications. Surgical sutures due to their wicking action can pull the bacteria and fluid into the wound site and pose a risk of developing SSIs.[10] In the literature, the use of antibacterial agents such as triclosan and chlorhexidine has been reported to coat the surgical sutures. In a systematic review, Wu *et al.* (2017) stated that antimicrobial sutures significantly reduced SSI risk.[11] The effect of antimicrobial coating was similar between different suture, wound, and procedure types. The quality of randomized controlled trials' evidence was moderate, and observational studies' evidence was very low quality. This systematic review concluded that TCS may reduce SSI risk; however, the available evidence is of moderate/low quality, and many studies had conflicts of interest. Thus, there is a paucity of data analyzing the effects of surgical sutures coated with these antibacterial agents in surgical periodontics. Hence, the present study was designed to evaluate how their antibacterial properties affect the tissues after periodontal flap surgery.

In the present study, there was no statistical difference between healing indices within all three groups. Thus, we can infer that the presence or absence of antibacterial coating on the suture does not affect wound-healing capacity of tissues. None of the patients developed any suture site infection during the follow-up period. Kruthi *et al.* reported that healing at the surgical site was slightly better on the 6th postoperative day in areas where TCS was used in comparison to NCS after minor oral surgical procedures.[12] Similar findings were recorded by Sharma *et al.* using CCS.[13] On the other hand, Rasić *et al.*[14] did not report any added benefit using antibacterial suture. Ford *et al.*[15] observed a decreased incidence of POP and diminished edema with the use of TCS as compared to standard NCS. However, in the current study, the use of antibacterial sutures did not alter the pain perceived by the patients.

On the other hand, visible plaque was reduced to 50% in TCS group as well as in CCS group, suggesting effectiveness of antibacterial-coated sutures in reducing plaque as compared to NCS. Wound dehiscence was observed in four patients of NCS suture group. While it occurred in two and three patients of TCS and CCS groups, respectively, on day 8 postoperatively, by day 15, adequate wound closure was seen in all patients of the three groups. This is in accordance with the findings reported by Kruthi *et al.*[12]

The absence of bacteria at the surgical site is one of the prime requirements for uneventful healing. Systemic antibiotics are used more commonly to avoid postsurgical infections. The use of antibiotics in immunocompromised patients is often required. However, injudicious use of antibiotics has led to emergence of antibiotic resistance. Moreover, with the use of
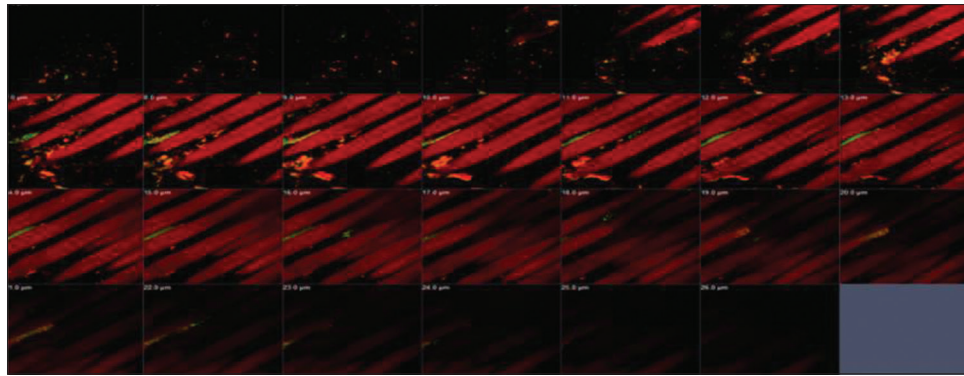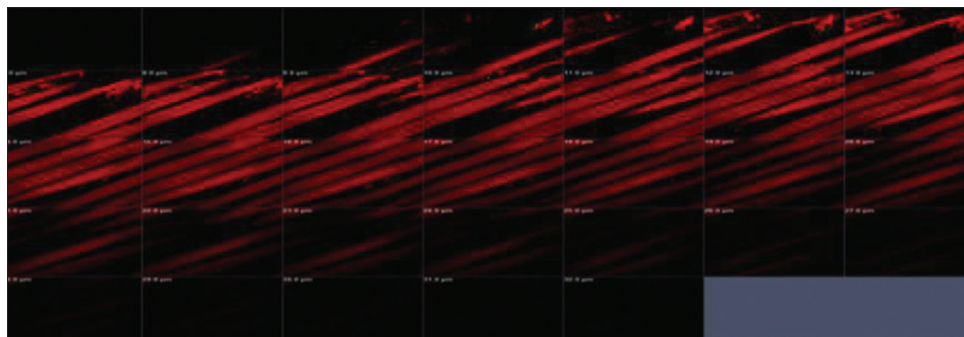
Figure 2: Noncoated suture series of two-dimensional confocal laser scanning microscopy images (z-stacks) of Live/Dead® (Syto-9 and PI) stained microbial biofilms on noncoated suture obtained at intervals of 1 μm (×100) magnification (green florescence [viable bacteria]) and (red florescence [nonviable bacteria])
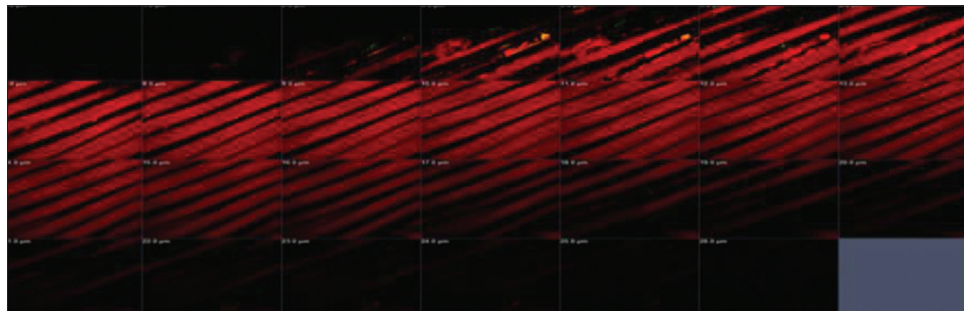
Figure 3: Triclosan-coated suture series of two-dimensional confocal laser scanning microscopy images (z-stacks) of Live/Dead® (Syto-9 and PI) stained microbial biofilms on triclosan-coated suture obtained at intervals of 1 μm (×100) magnification (green florescence [viable bacteria]) and (red florescence [nonviable bacteria])

Figure 4: Chlorhexidine-coated suture series of two-dimensional confocal laser scanning microscopy images (z-stacks) of Live/Dead® (Syto-9 and PI) stained microbial biofilms on chlorhexidine-coated suture obtained at intervals of 1 μm (×100) magnification (green florescence [viable bacteria]) and (red florescence [nonviable bacteria])

systemic antibiotics, local concentration of certain drugs fail to reach minimum inhibitory concentration for pathogens, thereby not effectively controlling their growth in oral cavity. Local delivery of antibiotics can be used to overcome these limitations. Antibacterial-coated suture is one of the effective alternatives to obtain a sustained release of antibacterial agent at the surgical site, thereby eliminating the need of systemic antibiotics. In our study, none of the patients reported any swelling or other signs of infections in spite of not receiving any systemic antibiotics. Similarly, Oswal *et al*. observed no postoperative infection in any of the study patients, irrespective of whether they received any prophylactic, therapeutic, or no antibiotic at all.[16]

Colony counts of aerobic as well as anaerobic bacteria were least in TCS followed by CCS whereas it was highest in NCS, and this difference was significant statistically ($P < 0.05$). Kruthi *et al*.[12]

reported that bacterial adherence was more in NCS as compared to TCS ($P < 0.001$). NCS group showed more of aerobic bacterial adherence whereas anaerobic bacteria were more adhered to coated suture groups. Sharma *et al*.[13] revealed that the aerobic bacteria load was higher in CCS as compared to NCS whereas the anaerobic bacterial load was more in NCS as compared to CCS. In our study, CCS group did not show statistically significant reduction in CFU count. This could be due to reduced drug concentration of the antimicrobial agent in the suture. Gram staining revealed the presence of Gram-positive cocci in clusters, Gram-positive and Gram-negative rods, Gram-positive filaments, and Gram-positive chains of cocci. Although specific bacterial species identification was not possible, based on the morphological characteristics and Gram staining, the colonies observed could be *Staphylococcus* species, *Streptococcus* species, *Escherichia coli*, *Actinomyces* species, and *Peptostreptococcus*

species. Alpha hemolysis observed around the colonies suggested the presence of viridians group of *Streptococci* species.

CLSM is a method to detect the presence of biofilm formation. Based on the specific color-coding system, one can visualize the presence of viable as well as dead bacteria in the biofilm formed. In the current experiment, CLSM detected the presence of bacteria not only on the surface of the suture material but also within the suture braids. In the present study, although CLSM showed the presence of biofilm formation on all the three types of sutures, the presence of viable bacteria was more on NCS as compared to TCS and CCS. In another *in vitro* study, the authors concluded that there was substantial reduction in biofilm formation on sutures coated with antibacterial agents.[9] Venema *et al.*[17] found that the numbers of green fluorescent, i.e., live bacteria, were similar on sutures with and without TCS. They concluded that TCS do not provide a sufficient antimicrobial effect to prevent *in vitro* colonization by oral bacteria. In our study, no significant benefit on wound healing was observed after the use of antibacterial sutures in periodontal flap surgery. TCS showed a significant reduction in the biofilm formation compared to plain NCS. In the current literature, a limited number of studies with small sample size and conflicting result make it difficult to draw any definitive conclusions. More well-defined randomized controlled studies with large sample size are needed to evaluate additional benefits antibacterial coating confers on the tissue-healing capacity. Furthermore, the exact concentration of the antibacterial agent and its drug release profile needs to be analyzed. In the present study, conventional access flap surgery was performed in all the patients who did not require any grafting material. However, further studies to evaluate the effect of antibacterial-coated sutures in other intraoral surgeries could be performed. The correlation between the use of such sutures and their effect on bone and soft-tissue loss postsurgery can further be studied. In our study, the characterization of the specific bacterial species within the biofilm around the sutures in all three groups could not be performed. Further studies, to generalize the results of the present study, should be carried out on a larger sample size.

CONCLUSION

Triclosan and chlorhexidine are known antibacterial agents. Local drug delivery in the form of coated sutures can be an effective method to inhibit biofilm formation and decrease the bacterial load at the surgical site as shown in our study, thereby reducing the need to give any systemic antibiotics and eliminating the need of antimicrobial mouthwash postsurgery. Moreover, the reduced biofilm formation near the surgical site can also improve the clinical success of any surgery. Thus, after analyzing and evaluating the data, it can be concluded that antibacterial sutures coated with either triclosan or chlorhexidine can be used in periodontal surgical procedure. However, its cost–benefit ratio should be evaluated in larger clinical trials to claim its dominance over conventional NCSs.

Conflicts of interest
There are no conflicts of interest.

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