Attachment of *Streptococcus Mutans* to Intraoral Suture Materials: An in Vitro Study

Denta Aditya Prasetya[1]*, Poerwati Soetji Rahajoe[2], Bambang Dwirahardjo[2], Michael Haryadi Wibowo[3]

1. Oral and Maxillofacial Surgery Residency Program, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia.
2. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia.
3. Department of Microbiology, Faculty of Veterinary, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Abstract

The aim of this study was to determine the amount of attachment of Streptococcuss mutans to suture materials such as nylon, polypropylene and triclosan-coated polyglactin 910 commonly used in intraoral sutures.

Nylon, polypropylene and triclosan-coated polyglactin 910, each of which consists of 12 samples with 3.0 in size, 1 cm long soaked for 72 hours in 100 µl of sterile saliva with S.mutans bacteria by 500 µl (1.5x 108 CFU / ml) and 1000 µl of Brain Heart Infusion broth. The amount of attached S. mutans was calculated by the plate count and Scanning Electron Microscope (SEM) methods. Data were analyzed with IBM SPSS 23 along with Anova test and Post-hoc test.

The plate count-based observation showed the amount of S. mutans attachment to nylon suture material was less than polypropylene (p = 0.019), lower than triclosan-coated polyglactin 910 (p = 0,000) where polypropylene was less than triclosan-coated polyglactin 910 (p = 0.002). The similar results were showed by SEM observation and resulted in an insignificant difference (p = 0.098) between nylon and polypropylene.

The study concluded that the lowest amount of attachment of S. mutans was found in nylon and the highest was in triclosan-coated polyglactin 910.

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Introduction

Removal of sutures in the intraoral suture will be performed on day 5-7, during which the bacteria will attach to the suture materials to allow the bacteria penetration into the wound. The bacteria causes the infection that later will further result in slow healing of the wound. *Streptococcus mutans* is one of the oral pathogenic bacteria with the ability to attach to hard surfaces connected by the pellicle (a thin layer of the matrix formed from salivary glycoproteins). Categorized as the gram-positive species-cocci, the bacteria dominate the formation of biofilms at the beginning of attachment.[1]. Ability to penetrate into the bloodstream and bind with fibrinogen to form bacterial and platelet molecular bridges for platelet aggregation, *S. mutans* are crucial in pathogenicity of endocarditis infection.[2]

The attachment ability of *S. mutans* bacteria is affected by the physical and chemical properties of the suture materials. Nylon, polypropylene and triclosan-coated polyglactin 910 are the materials that are commonly used in intraoral sutures today. Being a monofilament with a smooth surface, nylon and polypropylene supports fewer attachments of bacteria when compared to multifilament. Triclosan-coated polyglactin 910 is a multifilament with a wider surface to be exposed to bacteria and has a non-shedding area that allows bacteria to attach to and form colonies.[3] This suture material is absorbable, and degradable through the hydrolysis process.[4]. Addition of triclosan as the antibacterial on triclosan-coated polyglactin 910 aims to inhibit the attachment of bacteria to be minimum as in monofilament.[5]

This study was aimed at comparing the amount of *S. mutans* attached to nylon,

Corresponding author:
Denta Aditya Prasetya
Department of Oral and Maxillofacial Surgery
Faculty of Dentistry, Universitas Gadjah Mada
Yogyakarta-Indonesia.
E-mail: poerwati_soetji_fkg@ugm.ac.id

polypropylene and triclosan-coated polyglactin 910 with the use of the plate count method and observation with Scanning Electron Microscope (SEM).

Materials and methods

Saliva sampling

Among thirty candidates, a subject with healthy clinical conditions and normal values on blood tests; routine hematology, hemostasis, kidney function, liver function and blood sugar status was selected. Intraoral status examination showed good oral hygiene status, free of caries and calculus, no shakiness was found in the teeth and no ulcers were found or inflammation in the oral cavity area and the subject was not under orthodontic treatment. A healthy clinical condition and a healthy intraoral status are needed to ensure that the saliva obtained has the same composition as healthy human saliva in general.[6]

The selected subject was instructed not to eat, drink and brush teeth 2 hours before saliva collection. Saliva was obtained through spitting stimulated with paraffin chewing, The saliva obtained was centrifuged at 4000 x g within 10 minutes, and supernatant was taken and sterilized by pressure filtration.[7] The obtained sterile saliva was distributed into 36 preparations in a microtube, each of which contains 100 µl for each sample of suture material.

Research sampling

Nylon, polypropylene and triclosan-coated polyglactin 910 (Ethicon, USA) sized 3.0 and 1 cm long were prepared, each of which consists of 12 samples which were then put into two groups of observations; the plate count method and Scanning Electron Microscope observations (SEM; JSM-6510LA, JEOL, USA) that comprises 6 samples each. The suspension preparation of *S. mutans* bacteria (ATCC 25175) and Brain Heart Infusion broth (BHI) as bacterial nutrition was made under the guidelines that refer to the American Society of Microbiology.

Research procedures

All samples of suture materials were soaked in a different microtube containing 100 µl of saliva, 500 µl of *S. mutans* bacterial suspension and 1000 µl of BHI broth. Incubation was carried out at 37 ° C for 72 hours under facultative anaerobic conditions in which a BHI broth replacement was performed every 24 hours. Each sample was subsequently soaked in 10 ml Phosphate-buffered Saline (PBS) for 1 minute and repeated 3 times. The procedure was aimed at cleaning the unattached bacteria and the BHI broth on the surface of the suture material.

The plate count method-based observation was initiated with the detachment of *S. mutans*. After being washed with Phosphate-buffered Saline (PBS), the suture material was placed in a new microtube that contains 1.5 ml of sterile distilled water. Vortex was done to the microtube for 1 minute, after which serial dilution was performed until it reached a dilution factor of 10^{-3}. Bacterial suspension from a tube with a dilution factor of 10^{0} to 10^{-3} was cultured to the media to make sure the BHI in a Petri dish be triple cultured with each dilution factor. Petri dish was isolated using parafilm and incubated at 37 ° C. The result was read after 24 hours by counting the number of *S. mutans* bacterial colonies bred using a colony counter.

The second observation was performed with Scanning Electron Microscope (SEM) at 10 kV 5000x magnification, before which the fixation was conducted using a 4% paraformaldehyde solution pH 7.4 for 30 minutes. Each sample was then soaked in 70% 80%, 95% alcohol and pure alcohol as a dehydration process for 5 minutes each and dried with air and polished with platinum coating.[8] Representing the area attached with bacteria, each sample was taken for the observation. JPG images obtained were then processed to obtain data on the percentage of bacteria attachment which were calculated by comparing the pixel width of the existing *S. mutans* with that of the visual field in the photo obtained from SEM.[9]

Statistical analysis

The data obtained were processed using IBM SPSS version 23.0 application (IBM Corp., Armonk, NY, USA). The Saphiro-Wilk and Levene's tests were performed for the normality test and homogeneity test respectively. The post-hoc and correlation tests were used to compare the amount of *S. mutans* attachment in 3 suture materials. The analysis worked in the confidence level by 95% ($\alpha = 0.05$).

Results

1. Calculation of the growth of *S. mutans*

The calculation of *S. mutans* colonies from a total of 18 samples of suture materials (6

samples each) showed the lowest number of bacterial growth in the nylon group ($\bar{x}$= 3602.5) followed by the polypropylene group($\bar{x}$= 17876.5) and highest was found in the triclosan-coated polyglactin 910 group ($\bar{x}$= 43407.4) (Table 1).

Data in all groups were normally distributed (p> 0.05) and not homogeneous (p = 0.011). Anova test results showed a significant difference in the number of bacterial growth between groups (p = 0.000).

Triclosan-coated polyglactin 910 significantly had a bacterial growth rate approximately 3 times higher than polypropylene (p = 0.002) and 12 times higher than nylon (p = 0,000). The polypropylene group also showed a significantly higher amount of growth compared to nylon (p = 0.019) (Figure 1a). The Spearman's test showed that there was a correlation between suture material and the amount of bacterial growth (p = 0,000) and the correlation coefficient r = 0.944 (Figure 1b), suggesting that the suture material significantly influenced the amount of *S. mutans* bacteria growth.

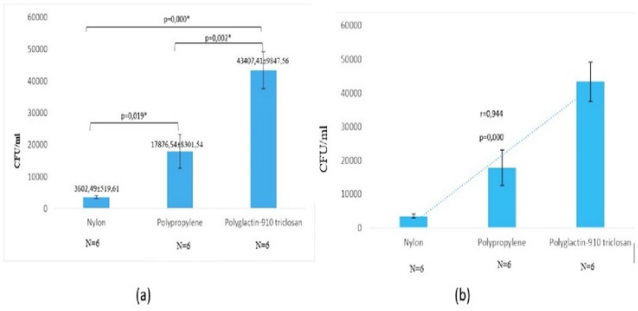

Figure 1: Different percentage of the growth of *S. mutans*), (a) Post Hoc Games-Howell test, (b) Correlation test.

2. Calculation of the percentage of attachment of *S. mutans*

Among 6 threads for the samples for the percentage of the bacterial attachment, 1 sample was found to be extreme data (outlier) in the polypropylene group. The extreme data occured because of the massive bacterial contamination beyond *S. mutans* possibly attached during the fixation process.

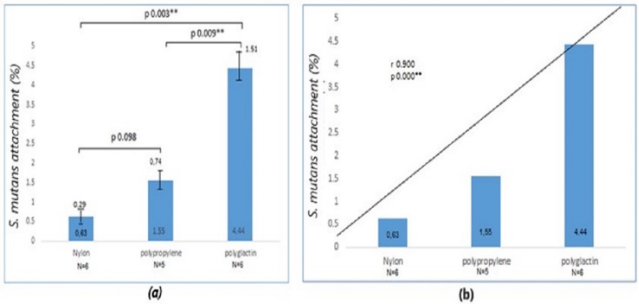

Figure 2: Different percentage of bacterial attachment (SEM), (a) Post Hoc Games-Howell test, (b) Correlation test

The observation results showed the lowest percentage of bacterial attachment in nylon ($\bar{x}$ = 0.63%) followed by polypropylene ($\bar{x}$=1.55%) and the highest in triclosan-coated polyglactin 910 ($\bar{x}$= 4.45%) (Table 1). Data in all groups were normally distributed (p> 0.05) and not homogeneous (p = 0.000). Anova results showed that there was a significant difference in the percentage of bacterial attachment between groups (p = 0.000).

Triclosan-coated polyglactin 910 significantly showed 7 times higher percentage than nylon (p = 0.003) and almost 3 times higher than polypropylene (p = 0.009). Polypropylene tends to show a higher percentage than nylon despite insignificance (p = 0.098) (Figure 2a). The Spearman's test showed a significant correlation between suture material and the percentage of bacterial attachment (p = 0,000) with a correlation coefficient r = 0.900 (Figure 2b), which means the type of suture material significantly affected the percentage of *S. mutans*.

Group n=6	Plate Count Method ($\bar{x} \pm SD$)	SEM Observation ($\bar{x} \pm SD$)
Nylon	3602.49 ± 519.61	0.63 ± 0,29
Polypropylene *)	17876.54 ± 8301.54	1.55 ± 0.74
Triclosan-coated polyglactin 910	43407.41 ± 9847.56	4.45 ± 1.51

Table 1. Observation results. *) n=5 in SEM Observation, because 1 sample was found to be extreme data (Outlier).

Discussion

Bacterial attachment in synthetic materials such as wound suture threads, prostheses and graft remain the objects of studies because of its important role in the mechanism of pathogenicity.[10] The low bacterial attachment to a suture material is the basis for selecting the suture material. This study compares the types of suture materials i.e. nylon, polypropylene and triclosan-coated polyglactin 910 with the use of plate count method and SEM observation. The observation results were generally similar; the lowest bacterial attachment was found in nylon, followed by polypropylene and triclosan-coated polyglactin 910 ($p = 0.000$) (Table 1).

The results of the plate count method showed that the triclosan-coated polyglactin 910 group had 12 times higher growth yield of *S. mutans* than the nylon group ($p = 0.000$) and 3 times higher than polypropylene ($p = 0.002$) (Figure 1a). A similar figure was found in the SEM observation that triclosan-coated polyglactin 910 had 7 times higher attachment percentage than the nylon group ($p = 0.003$) and almost 3 times higher than the polypropylene group ($p = 0.009$) (Figure 2a). Nylon and polypropylene have a monofilament structure. As a result, no non-shedding surface to be attached to bacteria. The degradation process did not occur in nylon and polypropylene, making the surface remain smooth to which bacteria find it hard to attach.

Several studies have proved that nylon and polypropylene are more superior than multifilament suture materials. Dragovic et al. (2017) used real time PCR and SEM to analyze the attachment of oral bacteria. The study concluded that polypropylene was significantly less attached by bacteria compared to silk multifilament suture materials.[11] Neto et al. (2015) stated that the level of total attachment of microorganisms in nylon was lower compared to multifilament suture materials such as silk, polyglactin 910 and triclosan-coated polyglactin 910, however, in certain types of bacteria such as *Provotella intermedia*, nylon has the same level of attachment as triclosan-coated polyglactin 910.[12] Especially on *S. aureus* bacteria, Masini et al. (2011) revealed that polypropylene also was less able to be attached than triclosan-coated polyglactin 910 despite insignificant difference.[10]

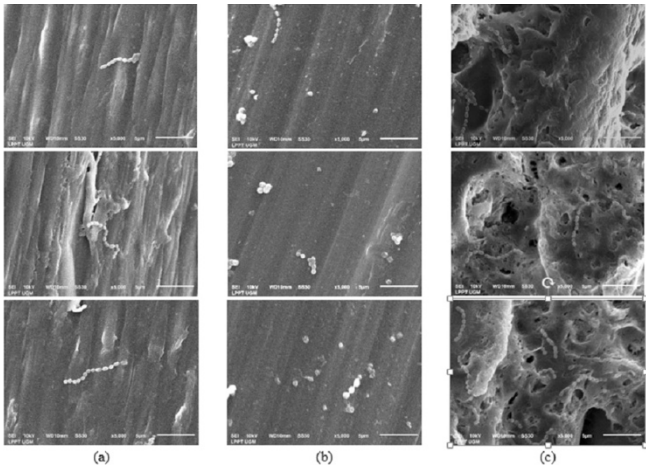

Figure 3. Attachment of Streptococcus mutans with a magnification of 5000 x (10kV), SEM observation on suture materials; (a) nylon, (b) polypropylene, (c) triclosan-coated polyglactin 910.

The addition of triclosan to polyglactin 910 is expected to inhibit the bacteria attachment to the suture material. Kruthi et al. (2014) proved that 40 patients who underwent oral surgery procedures and added triclosan to the polyglactin 910 significantly reduced the attachment of anaerobic bacteria compared to the one without triclosan.[13] Triclosan can inhibit the attachment of Staphylococcus aureus and Escherichia coli, exluding other types of bacteria such as *Staphylococcus epidermidis, Enterococcus faecium* and *Pseudomonas aeruginosa* which are insensitive to triclosan added to polyglactin 910.[14]

This study observed that high attachment of *S. mutans* to triclosan-coated polyglactin 910 indicated proved ineffective addition of triclosan to inhibit the attachment of *S. mutans*. The finding was also reinforced by in vitro study of Marzo et al. (2008) which proved that added triclosan to polyglactin 910 inhibits *P. aeruginosa* more effectively than *S. mutans*.[15] Triclosan is a broad-spectrum anti-microbial commonly used in the oral cavity and has a Minimum Inhibitory Concentration (MIC) against *S. mutans* bacteria at 7 , 8 mg / ml.[16] The lower concentration of MIC results in reduced effectiveness of triclosan in inhibiting bacterial attachment.[17] Furthermore, immersion within 72 hours may allow triclosan to dissolve in the suture material to reduce the concentration of triclosan.

A number of studies on antimicrobials found that antimicrobials smaller than MIC can modulate the biological characteristics of bacteria including

their ability to colonize.[18] Despite smaller than MIC, triclosan content in *S. mutans* can increase comD and luxS production which affect the mechanism of quorum sensing systems.[17] The comD gene is a histidynecinase sensor protein for competence-stimulating peptide (CSP) which increases the formation of bacterial colonies, while LuxS produces autoinducer 2 for biofilm formation. The increase in these two components ultimately result in more number of formation of *S. mutans* colonies in triclosan-coated polyglactin 910.[19]

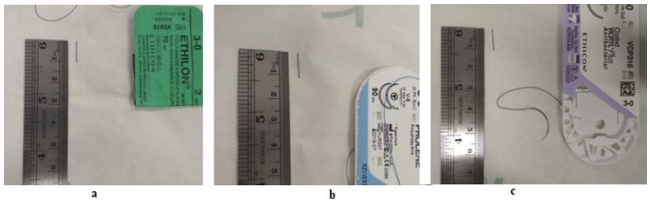

Figure 4. Samples of 3.0-sized and 1 cm-long suture materials, made by Ethicon, USA, (a) nylon, (b) polypropylene, (c) triclosan-coated polyglactin 910.

Triclosan-coated polyglactin 910 has a multifilament structure with strands of several threads resulting in a non-shedding surface for the bacteria to attach to and form colonies. Being absorbable, triclosan-coated polyglactin 910 make the surface degradable through the hydrolysis process caused by saliva and wet environmental condition.[4] The process occurs gradually and produces a non-shedding surface which makes the physical structure of this multifilament the perfect place for the bacteria to attach to and form colonies.

Under the chemical formula 6[NH-CO-$(CH_2)_5$]$_n$, nylon is a type of polyamide composed of long-chain aliphatic polymers[20] because of which nylon has hydrophilic properties while polyropylene with the chemical formula $(C_3H_6)_n$ is more hydrophobic. The different properties affect the ability of bacteria to attach to, but no studies have been done to compare the two. This study proves that nylon has a lower amount of attachment of *S. mutans* compared to polypropylene. The plate count method found a significant difference is ($p = 0.006$) but observations with SEM found the opposite ($p = 0.098$). This difference can occur due to different bacterial calculations. To calculate bacteria, the plate count method refers to the viable bacteria that can still multiply and form colonies, while SEM observation calculates all bacteria that are actually still attached to the suture material.[21] Despite different significance value, both methods still show similar results that nylon is less attached to *S. mutans* compared to polypropylene.

The attachment reaction of S. mutans biofilm to the matrix material is a complex process.[22] The hydrophobic nature causes polypropylene an easy target for the attachment of *S. mutans.* bacteria with a high to moderate hydrophobic surface.[23] Influenced by the hydrophobic nature of the microorganism cells, thermodynamics contributes considerably in the attachment process.[24] hydrophobic cells will attach more strongly to a hydrophobic surface.[25]

Correlation test shows a relationship between the type of suture material and the amount of bacterial attachment which proves that the type of suture material influences the amount of the attachment. Different results will be found in an in vivo study bacause this in vitro study was carried out by controlling substrate and environmental factors different from the actual condition of the oral cavity.

Diverse types of microorganisms in the oral cavity will also affect different attachment patterns due to the antagonistic or protagonistic nature of different kinds of microorganisms against *S. mutans* in forming biofilms to support their attachment and growth.[26] Gram positive coccus bacteria like *S. mutans* have higher peptidoglycan concentration that related to structural bacterial cell wall.[27] The other existing microorganisms such as *Candida albicans* can support the formation of *S. mutans* colonies which is coomon in the child's oral cavity while other bacteria such as Streptococcus sanguinis and *Streptococcus gonrdonii* which secrete H_2O_2 can inhibit the growth of *S. mutans*. In other interactions, *S. mutans* that produce lactic acid can also ultimately inhibit the growth of *Veillonella parvula*, the other oral cavity bacteria.[19] The complex interactions between these oral cavity microorganisms definitely require further clinical studies to prove the bacterial attachment to the suture material used in the oral cavity.

Conclusions

The study concluded that the lowest amount of attachment of *S. mutans* was found in

nylon and the highest was in triclosan-coated polyglactin 910. One of the main limitations of the study was that the various condition environtment in human oral is differences with in vitro. However, we are focused on carrying out additional experiments, taking into consideration various parameters and carrying out experiments in vivo in the future

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This manuscript is based on the thesis by author Denta Aditya Prasetya. All authors gave their final approval and agree to be accountable for all aspects of the work.

Declaration of Interest

The authors declare no conflicts of interest.

Acknowledgments

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