

Differences in denture brush fibers made of apus bamboo (*Gigantochloa apus*) and nylon based on the level of *Streptococcus mutans* contamination: an experimental study

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ABSTRACT

Introduction: Streptococcus mutans is one of the primary microorganisms responsible for plaque formation on dentures. Plaque is typically removed using a denture brush fibers made of nylon; however, nylon lacks antibacterial properties and may serve as a medium for bacterial contamination. As an alternative, natural fibers such as apus bamboo (Gigantochloa apus) may offer antibacterial benefits. This study aimed to analyze differences in denture brush fibers made of apus bamboo (Gigantochloa apus) and nylon based on the level of Streptococcus mutans contamination. Methods: The type of research was laboratory experimental with pre-test and post-test with control group design. Sample size was determined by the unpaired numerical comparative test sample size formula. Ten samples were assigned to the treatment group (apus bamboo fibers) and ten to the control group (nylon fibers). Each sample was immersed for three minutes in a bacterial suspension, followed by dilution to 10⁻⁵, and analyzed using the total plate count (TPC) method. Variable measurements were taken before and after 24 hours of standing, then the Streptococcus mutans contamination levels were compared between bamboo and nylon fibers. Results: The mean number of Streptococcus mutans contamination after 24 hours of standing on the bamboo fibers was 7.6×10^6 and on nylon fibers 1.7×10^5 . Mann-Whitney testing revealed a statistically significant difference between the two groups (p=0.009; p<0.05). Conclusion: There was a significant difference in Streptococcus mutans contamination levels between denture brush fibers made of apus bamboo (Gigantochloa apus) and nylon after standing for 24 hours.

Keywords

Brush fiber, dentures, bamboo apus, nylon, Streptococcus mutans.

Perbedaan serat sikat gigi berbahan bambu apus (Gigantochloa apus) dan nilon untuk gigi tiruan berdasarkan tingkat kontaminasi Streptococcus mutans: studi eksperimen

ABSTRAK

Pendahuluan: Streptococcus mutans merupakan salah satu mikroorganisme pembentuk plak pada gigi tiruan. Plak umumnya dapat dibersihkan menggunakan serat sikat gigi berbahan nilon, yang tidak memiliki efek antibakteri dan beresiko menjadi media kontaminasi. Serat nilon pada sikat gigi untuk gigi tiruan dapat digantikan oleh serat alam, yaitu bambu apus (Gigantochloa apus). Tujuan dari penelitian adalah untuk menganalisis Perbedaan serat sikat gigi berbahan bambu apus (Gigantochloa apus) dan nilon untuk gigi tiruan berdasarkan tingkat kontaminasi Streptococcus mutans. **Metode:** Jenis penelitian yaitu eksperimental laboratorik dengan rancangan pre-test and post-test with control group design. Pengambilan sampel menggunakan rumus besar sampel uji komparatif numerik tidak berpasangan. Kelompok perlakuan dengan serat bambu apus sebanyak 10 sampel sedangkan kelompok kontrol dengan serat nilon sebanyak 10 sampel. Setiap sampel direndam selama 3 menit di dalam suspensi, dilanjutkan dengan pengenceran hingga 10⁻⁵, dan dihitung dengan metode total plate count (TPC). Pengukuran variabel dilakukan sebelum dan setelah didiamkan 24 jam, kemudian jumlah kontaminasi Streptococcus mutans dibankan 24 jam pada serat bambu apus dan serat nilon. **Hasil:** Rerata jumlah kontaminasi Streptococcus mutans setelah didiamkan 24 jam pada serat bambu apus adalah 7.6x10⁶ dan serat nilon 1.7x10⁶, kemudian dilakukan pengujian menggunakan uji Mann-Whitney dan didapatkan nilai p=0,009 (p<0,05). **Simpulan:** Terdapat perbedaan antara serat sikat gigi tiruan bambu apus dan nilon berdasarkan tingkat kontaminasi Streptococcus mutans setelah didiamkan 24 jam.

Kata Kunci

Serat sikat gigi, gigi tiruan, bambu apus, nilon, Streptococcus mutans

INTRODUCTION

Tooth loss is one of the most prevalent oral health problems worldwide, affecting not only facial aesthetics but also chewing efficiency, speech, and overall quality of life. The etiology of tooth loss varies widely, including periodontal disease, dental caries, trauma, aging. Although prevalence increases with age, tooth loss is also observed in younger individuals due to habits such as smoking or poor oral hygiene. Untreated tooth loss can cause structural changes in the oral cavity and lead to additional complications, including temporomandibular joint disorders and reduced jaw bone density.^{1,2}

Tooth loss substantially increases the demand for dentures as a replacement for missing teeth. According to the 2018 Riskesdas data, 4.7% of the Indonesian population used removable dentures, whereas only 0.8% used fixed dentures. This preference may be driven by factors such as lower cost and ease of installation and maintenance. However, despite their advantages in flexibility and affordability, removable dentures present several challenges that must be considered, particularly concerning the long-term effects on oral health.^{3–5}

Prolonged use of removable dentures can impede the natural cleansing functions of the tongue and saliva in the oral cavity.³ This has the potential to cause accumulation of food debris on denture surfaces that are difficult to clean with conventional methods. This environment facilitates the colonization of opportunistic microorganisms, such as bacteria and fungi, which may cause various oral health problems, including stomatitis and other oral infections. Therefore, denture wearers should not rely solely on self-care, but also undergo regular dental check-ups to ensure that the dentures remain clean and free from microbial accumulation. Proper maintenance and awareness of the associated risks can help prevent complications and promote oral health.^{6,7}

Among microorganisms found in the oral cavity, particularly in denture wearers, *Streptococcus mutans* is one of the first bacteria to colonize denture surfaces and plays an central role in plaque formation.⁸ Plaque accumulation is not only aesthetically undesirable but also detrimental to oral health. If not properly removed, plaque may induce inflammation of the mucosal tissue under the denture, leading to denture stomatitis. This condition characterized by pain, redness, and swelling that compromise denture comfort.^{9,10}

Removable dentures can be cleaned mechanically, chemically, or by combining both methods. ¹¹ Mechanical cleaning using a denture brush is a simple and effective way to remove plaque. ¹² Denture brush fibers can be composed of either synthetic natural materials. ¹³ Nylona common synthetic fiber, is durable but lacks antibacterial properties, making it susceptible to bacterial contamination. ¹⁴ Bacteria adhering to brush fibers can multiply, and subsequently transfer to dentures, posing a risk of reinfection. ¹⁵

Natural fibers are increasingly being investigated as sustainable alternatives due to their eco-friendly characteristics and potential antibacterial components. One promising natural source is apus bamboo (*Gigantochloa apus*),^{15,16} a plant that is easy to cultivate and cost-effective. It is abundant in regions such as Ciawitali, Cimahi, making it a readily available material.¹⁷ Furthermore, apus bamboo fibers possess several advantages over conventional nylon fibers. They are lightweight yet strong, exhibiting high tensile strength and flexibility across applications.¹⁸ Its intrinsic antibacterial properties further enhance its value, making it a promising material for various purposes, including in the manufacture of products that require a high level of hygiene.¹⁹

This study was conducted to compare the effectiveness of denture brush fibers made of apus bamboo (*Gigantochloa apus*) and nylon, specifically in terms of *Streptococcus mutans* contamination levels. The results of this study are expected to provide new insights in the development of more hygienic and environmentally friendly denture brush products, offering a safer alternative for users. This study aimed to analyze differences in denture brush fibers made of apus bamboo (Gigantochloa apus) and nylon based on the level of Streptococcus mutans contamination.

METHODS

This study employed a laboratory experimental design with a pre-test–post-test control group format. Within this design, variables were measured before and after treatment, then the levels of *Streptococcus mutans* contamination were compared between bamboo fiber (test group) and nylon fiber (control group). The research was conducted in the Laboratory of the Faculty of Engineering, Jenderal Achmad Yani University, for the preparation of bamboo fibers, and at the Microbiology Laboratory of the Faculty of Medicine, Jenderal Achmad Yani University, for the preparation of *Streptococcus mutans* suspensions and the evaluation of *Streptococcus mutans* contamination on denture brush fibers.

Sampling in this study employed a purposive sampling method to ensure that each sample met predetermined characteristics. This study utilized a total of 20 samples, divided into two groups, one group was tested before being left for 24 hours, and the other after. Each group consisted of 5 nylon samples and 5 bamboo samples. The variables in this study were divided into two, namely the independent variable (type of fiber: bamboo or nylon) and the dependent variable (Streptococcus mutans contamination level).

The research procedures included making bamboo fiber brushes, preparing nylon fibers, sterilizing tools and materials, and measuring *Streptococcus mutans* contamination. *Streptococcus mutans* cultures were rejuvenated and placed into a test tube containing sterile 0.9% NaCl, then homogenized using a vortex mixer. The turbidity of the bacterial suspension was visually equalized to the McFarland 0.5 standard. Bamboo fiber and nylon fiber samples were immersed for 3 minutes in 150 ml of *Streptococcus mutans* suspension and subsequently rinsed using 0.9% NaCl for 10 seconds.

Bamboo and nylon fibers were divided into two treatments. In the first treatment, the rinsed fibers were placed into a beaker glass containing 0.9% NaCl and then placed on a vortex machine for 1 minute, to detach Streptococcus mutans bacteria adhered to the fiber surface. A 1 mL aliquot of the vortexed NaCl suspension was collected using a micropipette and serially diluted (10^{-1} to 10^{-5}) in test tubes, each containing 9 ml of NaCl, then shaken until homogeneous.

The results of the dilution were plated by transferring 1 ml of each dilution into Petri dishes along with plate count agar media that had not yet solidified (45°C). The mixture was gently swirled for uniform distribution and allowed to solidify. The solidified media were incubated at 37°C for 24 hours. Bacterial colonies were then counted using a colony counter, and the results were expressed as colony-forming units per milliliter (CFU/mL). In the second treatment, the rinsed fiber samples were placed in sterile beakers, covered with plastic wrap, and left to stand for 24 hours at room temperature. After this period, they were vortexed and processed for colony counting following the same procedure as in the first treatment.

Data analysis was performed using the Statistical Product and Service Solution (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA) application. The normality of the data was assessed using the Shapiro-Wilk test (n<50). For normally distributed data, paired t-tests were used to compare *Streptococcus mutans* contamination levels before and after 24 hours of standing within each fiber type (apus bamboo and nylon). Subsequently, an unpaired t-test was conducted to determine whether there was a significant difference in *Streptococcus mutans* contamination between denture brush fibers made of apus bamboo (Gigantochloa apus) and nylon. If the data were not normally distributed, the Mann Whitney and Wilcoxon statistical tests were used as non-parametric alternatives.

RESULTS

Descriptive data processing was performed to characterize the Streptococcus mutans contamination levels on denture brush fibers made of apus bamboo (Gigantochloa apus) and nylon. The descriptive statistics for both fiber types are presented in Table 1.

Table 1. Descriptive analysis of denture brush fibers made of apus bamboo (Gigantochloa apus) and

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Variables	Mean	Media n	SD	Min-Max
Bamboo fiber without being standing	1.6x10 ⁶	1.6x10 ⁶	4x10 ⁵	1.2x10 ⁶ -2.2x10 ⁶
Bamboo fiber after being left for 24 hours	7.6x10 ⁶	3.2x10 ⁶	$7.9x10^6$	2.6x10 ⁶ -2.1x10 ⁷
Nylon fiber without being standing	2.9x10 ⁵	2.7x10 ⁵	1.5x10 ⁵	1.4x10 ⁵ -5.4x10 ⁵
Nylon fiber after being left for 24 hours	1.7x10 ⁵	1.8x10 ⁵	2.5x10 ⁴	1.5x10 ⁵ -2.1x10 ⁵

Based on table 1, the bacterial contamination on brush fibers ranged from a minimum of 1.2×10^6 CFU/mL in bamboo fibers before standing, to a maximum of 2.1×10^7 CFU/mL in bamboo fibers after being left for 24 hours.

As shown in Table 2, there was a significant difference (P<0.05) in the mean *Streptococcus mutans* contamination levels before and after 24 hours of standing in denture brush fibers made of bamboo. A normality test using Shapiro-wilk (n<50) revealed that the data before 24 hours of standing were normally distributed, while the data after 24 hours were not normally distributed. Consequently, the non-parametric Wilcoxon test was used to analyze differences in Streptococcus *mutans* contamination levels for denture brush fibers made of bamboo. The bacterial contamination of *Streptococcus mutans* on the denture brush fibers made of bamboo can be seen in Table 2.

Table 3. Streptococcus mutans contamination on denture brush fibers made of nylon						
Types of denture	The average value of the number of Streptococcus mutans contamination (CFU/ml)					
brush fibers	Without being standing	After being left to stand 24 hours	P Value			
	n=5	n=5				
Nylon Mean±Std	2.9x10 ⁵ ± 1.5x10 ⁵	1.7x10 ⁵ ± 2.5x10 ⁴	0.156			

As shown in Table 3, there was no significant difference (P>0.05) in the mean S. mutans contamination levels of denture brush fibers made of nylon before and after 24 hours of standing. The normality test using Shapiro–Wilk indicated that both datasets were normally distributed; therefore, the paired t-test was applied to compare contamination levels. The contamination of *Streptococcus mutans* bacteria on nylon brush fibers can be seen in Table 3.

Table 4. Differences in *streptococcus mutans* contamination on denture brush fibers made of bamboo and nylon

Treatment	The average value of the mutans contam	D.V-I	
Treatment	Bamboo apus n=5	Nylon n=5	- P Value
After being left for 24 hours			
Mean±Std	$7.6 \times 10^6 \pm 7.9 \times 10^6$	$1.7 \times 10^5 \pm 2.5 \times 10^4$	0.009*

As presented in Table 4, the mean S. mutans contamination after 24 hours of standing differed significantly between denture brush fibers bamboo and nylon (P<0.05). The Shapiro—

Wilk normality test showed that nylon fiber data were normally distributed, whereas bamboo fiber data were not normally distributed. Therefore, the Mann-Whitney test was applied as a non-parametric alternative to assess the difference in S. mutans contamination between the two fiber types. The difference in the level of contamination of *Streptococcus mutans* bacteria between bamboo apus and nylon fibers as denture brush fibers after 24 hours of standing can be seen in table 4.

DISCUSSION

The bacteria *Streptococcus mutans* was detected in both denture brush fibers made of bamboo and nylon, as shown in Table 1, consistent with the findings of Raiyani et al.²⁰ *Streptococcus mutans* exhibits greater resilience to unfavorable environmental conditions compared with other oral bacteria, such as *Streptococcus sanguinis*. This resilience is attributed to several virulence factors, including acid production, extracellular polysaccharide synthesis, and surface adhesin development.²¹

Adhesins allow bacteria to attach to fibrous surfaces and proliferate through transverse binary fission, an asexual reproduction process that generates genetically identical daughter cells. The reproduction process of *Streptococcus mutans* is time-dependent, meaning that the longer the denture brush fiber is in contact with *Streptococcus mutans*, the greater the bacterial proliferation.⁸ Contaminated brush fibers can serve as a source of reinfection and pose potential risks to oral health, as microorganisms may proliferate on the fibers, migrate to denture surfaces, and transmit to users upon reuse.²²

As presented in Table 2, the mean number of *Streptococcus mutans* colonies on bamboo fibers increased after 24 hours of standing. This observation contrasts with the findings of Yoshimasa et al., who reported a 95–98% reduction in *Streptococcus mutans* counts on bamboo brush handles after 24 hours.²³ Bamboo toothbrushes produced commercially often have bamboo handles but nylon bristles; therefore, differences in fiber materials may influence bacterial retention after drying and standing. Nylon fibers are minimally affected by moisture, whereas bamboo fibers are highly moisture-sensitive, particularly after prolonged exposure.^{24,25}

In this study, denture brush bristles were left to stand for 24 hours post-rinsing, following Ramful's findings that the optimal antibacterial properties of bamboo peak between 24 to 48 hours. Bamboo culms primarily consist of cellulose, hemicellulose, and lignin. The chemical composition of lignin, including phenolic compounds, carboxylic acids with hydroxyl groups, and oxygen-containing methoxyl and epoxy functional groups, is a critical factor influencing its antibacterial activity. Yun et al. demonstrated that increased hydroxyl group concentrations in lignin effectively inhibited the growth of *E.coli* (95.61%), *Salmonella* (89.60%), *Streptococcus* (66.62%), and *S.aureus* (64.68%).

The antibacterial mechanism of lignin is primarily attributed to its ability to create a low pH environment, disrupting the proton balance across bacterial cell membranes. Lignin-derived polyphenols can damage bacterial cell walls through lysis, causing leakage of intracellular fluids in bacteria. Additionally, lignin produces reactive oxygen species (ROS) that are concentrated on the cell surface when it comes into contact with bacteria, but there is no research on how specific ROS can be produced by lignin on bamboo. ROS can cause oxidative stress by altering normal redox physiological processes in bacterial cells. The higher the lignin content in bamboo fiber, the more the bacterial cell wall will be damaged and cause bacterial death.^{26,27}

Bamboo also contains hemicellulose, cellulose and parenchyma that influence its moisture content. Parenchyma cells have high water-binding capacity, which will affect the strength and durability of the resulting bamboo fiber. Bamboo fiber for brush making is obtained from the middle layer of the bamboo stem, which contains the highest parenchyma concentration, resulting in greater water absorption.²⁸ The thickness of the tissue in bamboo will also affect the capacity of the cells to hold water.

Bamboo has a greater thickness at the base than the center and top, so the highest water absorption in bamboo is at the base of the stem. The hemicellulose content affects the water

release dynamics. These properties collectively contribute to higher moisture content and, consequently, greater bacterial colonization. Furthermore, bamboo also has pores that are shaped like cavities. The more pores in bamboo, the lower the density value of bamboo, which is known to be 0.60 (g/cm3), this low density value in bamboo facilitates moisture accumulation. The higher the moisture content in bamboo fibers, the lower the strength and hardness of the fibers, causing bamboo fibers to become brittle and break easily.²⁹

In contrast, there was no significant difference in *Streptococcus mutans* contamination between nylon fibers before and after 24 hours of standing although a slight reduction was observed (Table 3). Nylon is a widely used materials for making brush fibers and does not have antibacterial properties.¹⁴ Nylon has a low water absorption rate of approximately 4%, which means nylon fibers dry quickly and provide less favorable environment for bacterial growth.^{25,30} Although bacterial counts decreased over 24 hours, they were not completely eliminated, consistent with Silva et al's research which found that *Streptococcus mutans* could survive on nylon fibers for 24 hours and began to disappear at 44 hours.³¹ Caroline et al similarly reported detectable *Streptococcus mutans* bacteria on brushes after 24 hours of drying.²¹ The persistence of *Streptococcus mutans* on nylon brush fibers is linked to the presence of extracellular polysaccharides serving as nutrient sources, which gradually diminish over, explaining the reduction in bacterial numbers.³¹

Environmental storage conditions also influence bacterial survival. Thalya et al. indicated that bacteria can persist on brushes for 24 hours, especially under humid conditions. ²⁴ Young Lee et al. indicated that *Streptococcus mutans* bacteria were present on brush fibers stored in bathrooms, but not in dry indoor settings. ³² Such humid environments can sustain bacterial survival, making brushes potential reservoirs for cross-contamination. ²⁴

A significant difference was observed in S. mutans contamination levels between apus bamboo and nylon fibers after 24 hours (Table 4), with bamboo exhibiting higher bacterial counts. This finding aligns with Srishti et al., 15 who reported that bamboo brush handles do not have significant antibacterial properties compared to nylon brush handles, so the amount of bacterial contamination is higher in bamboo brush handles. 15 Avaneethram et al similarly found that *Candida* can grow 76% higher on bamboo brush fibers than on nylon brush fibers. 33

However, other studies provide contrasting evidence. Nayak et al.³⁴ reported that bamboo brushes exhibit antibacterial capabilities and are environmentally friendly due to their biodegradable characteristics.³⁴ While Venkatasubramanian et al observed lower bacterial contamination in bamboo handles when treated with castor oil, which reduces microbial adherence.³⁵

The difference in contamination levels may also be influenced by the electrostatic characteristics of nylon. *Streptococcus mutans* attached to nylon fibers may not be fully dislodged during vortex processing. Matayoshi et al found that about 40-55% of *Streptococcus mutans* cells remained attached on the fibers after applying vibration using a vortex machine.¹⁴

Caroline et al.,³⁵ reported that variations in brush fiber design can influence bacterial retention; factors such as bristle count, length, surface roughness, and microscopic defects may create niches that facilitate bacterial accumulation.³⁵ In this study, the bamboo fiber used was 1.5 mm in diameter due to the limitations of bamboo fiber, which has a rather stiff and brittle nature, while the diameter of nylon fiber can reach 0.2 mm with soft characteristics. Brush fibers with softer characteristics tend to have lower bacterial retention than stiffer, brittle fibers.³⁶

Differences in water absorption between bamboo and nylon fibers influence the degree of *Streptococcus mutans* contamination. Bamboo fiber exhibits a water absorption rate of 7.7%, which is relatively high and classifies it as a hydroscopic material, in contrast to nylon fiber, which demonstrates a considerably lower water absorption rate of around 4%.^{25,30} The lower water absorption of nylon fibers contribute to greater structural resistance and reduced bacterial growth compared with natural fibers such as bamboo.¹⁴ Conversely, the high water absorption capacity of bamboo fibers promote an environment conducive to bacterial growth.³³ The addition of certain materials such as surface pre-reacted glass-ionomer (S-PRG) fillers, or

applying suitable processing modifications, may enhance the mechanical strength of bamboo fibers and reduce their water absorption capacity, thereby minimizing bacterial growth.

CONCLUSION

The level of *Streptococcus mutans* contamination on denture brush fibers made of bamboo increased significantly after 24 hours of standing, with the highest mean bacterial count recorded at 7.6x10⁶ CFU/mL. In contrast, denture brush fibers made of nylon exhibited no significant change before and after 24 hours. There is a difference between denture brush fibers made of bamboo and nylon based on the level of *Streptococcus mutans* contamination after standing for 24 hours. The results of this study advocate for the development of natural fiber materials with improved resistance to bacterial contamination and advancing processing technologies designed to mitigate microbial contamination. Although natural materials such as bamboo provide environmental advantages, maintaining optimal health and hygiene standards remains essential when selecting materials for toothbrushes.

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Data Availability Statement: The availability of research data will be given permission by all researchers via email correspondence with due observance of ethics in research.

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