Comparison of bacterial contamination in bristles of charcoal toothbrushes versus non-charcoal toothbrushes

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WHY THIS ARTICLE IS IMPORTANT TO DENTAL HYGIENISTS

- Micro-organisms have been shown to adhere to and survive on toothbrushes.
- Bacterial contamination of toothbrushes contributes to oral diseases.
- Identifying materials that reduce bacterial contamination of toothbrush bristles may improve oral health.

ABSTRACT

Objective: Charcoal toothbrushes have been marketed widely with manufacturers' claims of lesser bacterial contamination owing to the presence of activated charcoal. The aim of this study was to evaluate the bacterial contamination of charcoal bristles compared to non-charcoal bristles in used toothbrushes. **Material and methods:** Ninety participants were involved in the study. They were given standard brushing instructions on the use of a charcoal toothbrush, and were asked to return the used brushes after 1 week of usage. After a 1-week washout period, the participants were then provided with similar brushing instructions and a non-charcoal toothbrush, and were instructed to return the brush after another week of usage. Bristles of the used toothbrushes were sectioned and placed in a nutrient broth. A pipette was used to extract 0.1 mL of nutrient broth to smear on agar plates. A colony counter was used to measure colony forming units (CFU) after 24 hours of incubation. Data collected were analysed using a paired sample t-test. **Results:** The mean CFU count for non-charcoal bristles was almost double (106.3; 95% CI 53.39, 159.28) that of charcoal bristles (58.8; 95% CI 15.09, 102.55). However, there was no statistically significant difference between the two groups (p = 0.198). **Conclusion:** This study shows no statistically significant difference in bacterial counts between bristle types, despite substantially lower CFUs in the charcoal bristles compared with non-charcoal bristles after 1 week of use.

RÉSUMÉ

Objectif : La mise en marché des brosses à dents au charbon a été largement axée par les fabricants sur la réduction de la contamination bactérienne en raison de la présence du charbon activé. La présente étude avait pour objectif l'évaluation de la contamination bactérienne des poils de charbon par rapport aux poils sans charbon des brosses à dents usagées. **Matériau et méthodes :** Quatre-vingt-dix participants ont pris part à l'étude. Les participants ont reçu les instructions habituelles de brossage sur l'utilisation d'une brosse à dents à poils de charbon et ont été invités à retourner les brosses à dents usagées après une semaine d'utilisation. Après une période sans traitement d'une semaine, les participants ont reçu des instructions de brossage semblables et une brosse à dents à poils sans charbon. Ils ont été invités à retourner la brosse après une autre semaine d'utilisation. Les poils des brosses à dents usagées ont été sectionnés et placés dans un bouillon de culture. Une pipette a été utilisée pour extraire 0,1 mL de bouillon de culture et l'étaler sur des plaques de gélose. Un compteur de colonies bactériennes a été utilisé pour mesurer les unités formatrices de colonies (UFC) après 24 heures d'incubation. Les données recueillies ont été analysées au moyen de test t pour échantillons appariés. **Résultats :** La concentration moyenne d'UFC présente sur les poils sans charbon était presque le double (106,3; 95 % CI 53,39, 159,28) de celle présente sur les poils de charbon (58,8; 95 % CI 15,09, 102,55). Toutefois, il n'y avait aucune différence statistiquement significative entre les deux groupes (p = 0,198). **Conclusion :** Cette étude ne révèle aucune différence statistiquement significative dans le compte de bactéries entre les types de poils, malgré la présence d'un nombre nettement plus faible d'UFC sur les poils de charbon comparativement aux poils sans charbon après une semaine d'utilisation.

Key words: bacterial contamination, charcoal bristles, used toothbrushes

INTRODUCTION

Toothbrushes become contaminated with pathogenic bacteria from dental plaque, the environment or a combination of factors. Mehta et al.[1] studied the effectiveness of various methods of reducing bacterial contamination of toothbrushes, including covering the toothbrush head with a plastic cap, overnight immersion of toothbrushes in Listerine®, and overnight immersion of brushes in chlorhexidine. Each method was tested for a 1-week

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Figure 1. Non-charcoal and charcoal toothbrushes used in this study

period. The results revealed that overnight immersion of a toothbrush in 0.2% chlorhexidine gluconate was more effective than overnight immersion in Listerine or covering the toothbrush head with a plastic cap.[1] This study also concluded that 70% of the used toothbrushes were heavily contaminated with different pathogenic microorganisms.[1] Several other studies have also investigated various methods of brush decontamination.[2-6]

A new variant of toothbrushes, charcoal toothbrushes, has been introduced into the market; these toothbrushes are popular in South-East Asian countries like Malaysia, Singapore, and Indonesia.[7] Consumers can also buy these products through online vendors.[7] Bristles of charcoal toothbrushes are black in colour and are prepared by blending binchotan charcoal into nylon bristles. Manufacturers of these toothbrushes claim that they have antimicrobial properties thanks to the charcoal in them, resulting in less bacterial contamination.[7] However, there is no scientific evidence to support these claims.

It has been well-established that micro-organisms adhere, accumulate, and survive on toothbrushes.[2] Furthermore, these microbes have been shown to be capable of transmission to the individual, which in turn can cause diseases.[8] Decontamination of toothbrushes should be a priority in order to eliminate the transmission of pathogenic micro-organisms from the oral cavity or from other toothbrushes stored nearby or from the storage area itself.[9] Various materials have been incorporated into toothbrush bristles with the aim of reducing bacterial contamination.[2] Since it has been suggested that charcoal may have bacterial resistant properties, toothbrushes have been created with charcoal infused into the bristles. The aim of this study was to evaluate the bacterial contamination of charcoal bristles compared to non-charcoal bristles in used toothbrushes by comparing the microbial counts present in the bristles.

Figure 2. Used charcoal and non-charcoal toothbrushes returned in sterile pouches

MATERIAL AND METHODS

This crossover clinical trial was approved by the Institutional Ethics Committee of SEGi University. Students who attended the SEGi Oral Health Centre from June 2015 to August 2015 formed the sampling frame. Those ages 18–25 years with toothbrushing frequency of 2 times daily were eligible for inclusion in the study. Students selected for the study had basic periodontal examination (BPE) scores of 1 and 2[10]; students with BPE scores of 3 and 4 were excluded. Likewise, students with International Caries Detection and Assessment system (ICDAS)[11] scores of ≥3 were excluded from the study. Students with open carious lesions, poor plaque scores (plaque index scores of >2),[12] severe gingivitis (gingival index score >2),[12] throat infections, irregular brushing frequency, as well as those unwilling to use a charcoal toothbrush, those using mouthwash and/or antibacterial toothpastes, smokers or those medically compromised were excluded from the study. All the students who participated in the study were manual brush users. From the name list of 200 students

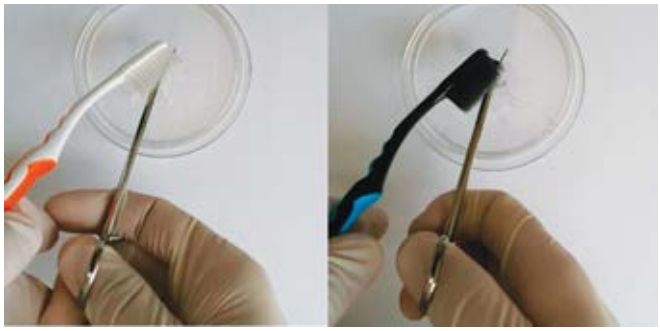

Figure 3. Bristles collected in sterile petri dishes

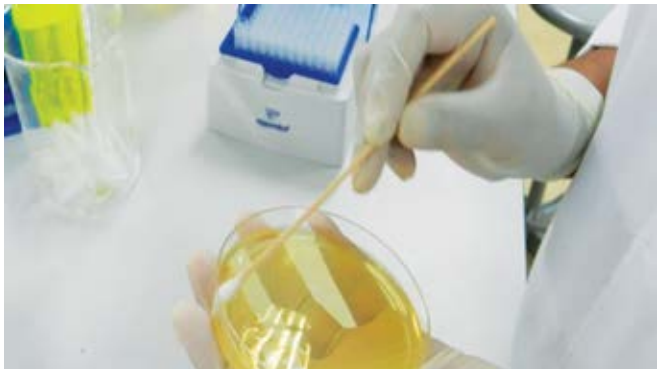

Figure 4. Nutrient broth containing used toothbrush bristles is smeared on the nutrient agar plate

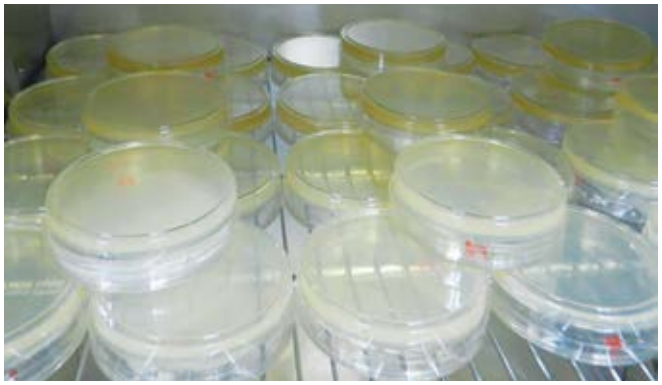

Figure 5. Smeared nutrient agar plates placed for incubation

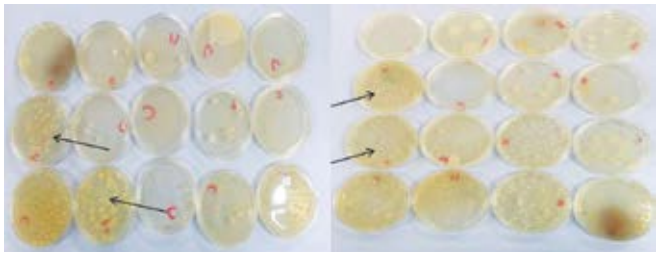

Figure 6. Microbial growth noticed after 24 hours incubation (plates marked "c" contain charcoal bristles; plates marked "n" contain non-charcoal bristles)

(provided by the course coordinator of the university) who met the inclusion criteria, 90 participants were randomly chosen. All 90 participants were informed about the study and signed the consent form prior to participation.

All participants were given standard instructions on toothbrushing and toothbrush storage to minimize bias in the study. Standard brushing instructions included brushing twice daily (once each in the morning and night) for 2 minutes.[13] Students were instructed to place the brush at a 45-degree angle to the gums and gently move the brush back and forth in short strokes. Participants were instructed to brush the outer surfaces, the inner surfaces, and the chewing surfaces of all teeth. They were also instructed to clean the inside surface of the front teeth, tilting the brush vertically and making several up-and-down strokes.[13] They were also advised not to use any type of mouthwash, to wash the toothbrush bristles under running water without using their fingers to clean the bristles, not to cover the toothbrush bristles with a cap, and to place the toothbrush upright after use with the bristles on top at least 2 feet away from the toilet. Researchers from the University of Alabama found that brushes stored in the bathroom are very likely to have faecal matter lingering in the bristles.[14] Toilet flushing was shown to produce an aerosol spray of bacterium tainted water which can contaminate the bristles.[14] Thus, study participants were instructed to keep the toothbrushes at least 2 feet away from the toilet. Students were asked to document their daily 2-minute brushings on a standardized recording sheet provided to them.

Each participant was then given a charcoal toothbrush and asked to return the toothbrush after 1 week of use. After a wash-out period of 1 week, non-charcoal toothbrushes were given to the participants and again, they were asked to use the brushes for 1 week and to return the non-charcoal toothbrushes after the week. Both the charcoal and non-charcoal brushes were similar in design with a compact head, soft bristles, and a bristle tip that was less than 0.01 mm (Figure 1; Colgate® Slim Soft Charcoal Toothbrush). The participants received individual sterile pouches into which to place each used toothbrush for return (Figure 2).

On return of the toothbrushes, one-third of the bristles were cut and collected on separate sterile petri dishes (Figure 3). Using sterile forceps, the study assistant placed the toothbrush bristles in separate test tubes containing a nutrient broth and swirled. A sterile pipette was used to extract 0.1 mL of the nutrient broth, which was poured onto a nutrient agar plate. A sterile cotton bud was used to smear the solution on the agar plate (Figure 4). The agar plates were then placed in the incubator for 24 hours (Figure 5), after which colonies of microbial growth were noted (Figure 6). Colony counters (Fisher Scientific brand, model F22 0360/10R) were used to measure the colony forming units (CFU) present on each agar plate

(Figure 7). Data obtained were tabulated and statistically analysed using MedCalc ver 12. A paired sample t-test was conducted to compare the number of CFUs for charcoal and non-charcoal bristles. The significance level was set at $p < 0.05$. Mean values for CFU counts and 95% confidence intervals for the mean were determined for the 2 groups.

RESULTS

Of the 90 participants, 3 did not return one of their toothbrushes. Five participants did not properly place their toothbrushes in the sterile pouches provided and these (5 x 2 brushes) were excluded from the study. A final count of 164 toothbrushes—82 charcoal and 82 non-charcoal—were collected from participants. Out of 164 agar plates (82 charcoal and 82 non-charcoal), 102 plates (51 charcoal and 51 non-charcoal) were seen to have microbial colonies and included in the analysis. There were no growths seen in 62 plates after 24 hours of incubation. Using the colony counters, higher counts of CFUs were seen on the agar plates from used non-charcoal brushes compared with those from used charcoal brushes.

Table 1 presents the results of the paired sample t-test comparing the number of CFUs between the 2 types of bristles. The mean CFUs for non-charcoal bristles were almost double (106.3; 95% CI 53.39, 159.28) those of the charcoal bristles (58.8; 95% CI 15.09, 102.55). However, there was no significant difference between the 2 products ($p = 0.198$).

DISCUSSION

Results revealed substantially lower CFU counts in agar plates for used charcoal bristles compared with used non-charcoal bristles. This difference, however, was not statistically significant. This is most likely due to the high variability of CFUs demonstrated by the standard deviations found in both products. A power analysis was not performed prior to study commencement. A post-study power analysis revealed a sample size of 209 brushes was required (alpha value of 0.05, beta value of 0.20) to obtain a statistically significant difference between means. To date, there is a dearth of scientific literature on toothbrushes with charcoal infused bristles. Manufacturers' claim that charcoal toothbrushes control micro-organisms, inhibit mouth odour, effectively remove plaque, and whiten teeth, yet such claims are not supported by scientific evidence on bacterial inhibition. Charcoal in itself has the property of being absorbent, neutralising toxins, poisons, and noxious gases.[3] However, it continues to be a matter of speculation as to whether these properties contribute to lesser contamination of used charcoal-infused bristles in toothbrushes.

Additions of antiplaque and antimicrobial substances to toothbrush bristles in attempts to reduce contamination of used toothbrushes are not a new phenomenon. Turner et al. conducted a study to determine the effectiveness of chlorhexidine-coated toothbrush filaments in reducing quantities of bacteria.[3] The study concluded that there was no statistically significant difference in the quantity of bacteria surviving on chlorhexidine-coated filaments compared with the control group after 30 days of use.[3] The manufacturer of the chlorhexidine-coated toothbrush, however, suggested that chlorhexidine-coated filaments were only effective for a 30-day period, after which time the toothbrush should be replaced.[3] Al–Ahmad et al. studied the antimicrobial effect of silver-coated toothbrush heads in-vitro.[4] The organisms investigated were *Streptococcus oralis, Streptococcus mutans, Streptococcus sanguis, Actinomyces viscosus, Lactobacillus casei* and *Candida albicans*. The study concluded that there was no significant reduction in the CFUs by silver-coated toothbrushes for the above-mentioned tested organisms.[4] On the contrary, the CFU counts for *S. sanguis* ($p = 0.02$) and *C. albicans* ($p = 0.01$) were significantly higher on silver-coated toothbrushes compared with the controls.[4] This current study did not investigate specific organisms; only microbial counts were made.

In 2014, Tomar et al. evaluated the sanitization potential of UV-rays and 0.2% chlorhexidine (CHX) solution for disinfection of used toothbrushes.[5] Toothbrushes were collected after 7 days of use and placed into 3 groups: Group I brushes were soaked in 0.2% CHX mouthwash

Table 1. CFU differences between charcoal and non-charcoal toothbrush bristles

	Used charcoal brushes n = 51	Used non-charcoal brushes n = 51
CFU mean (SD)	58.8235 (155.48)	106.3333 (188.23)
Standard error of the mean	21.7720	26.3580
Mean difference (SD)	47.5098 (259.92)	
95% CI	–25.5938 to 120.6134	
2-tailed probability	$p = 0.198$	

Paired sample t-test significant if $p < 0.05$

for 12 hours, Group II brushes were placed in UV-light toothbrush holders for 7 minutes, and Group III brushes were soaked in normal saline for 12 hours. Microbial analysis and mean bacterial counts showed that all 3 methods were effective in reducing the bacterial counts on the toothbrushes tested ($p < 0.007$). However, UV ray treatment was more effective ($p = 0.001$) when compared with CHX and normal saline.[5] The authors suggested that UV light is capable of deactivating the micro-organisms by disrupting the chemical bonds that hold the DNA atom.[5] Studies have suggested that longer exposure to UV light can further lead to complete deactivation of micro-organisms.[5]

Basman et al. studied toothbrush disinfection using 0.12% chlorhexidine gluconate, 2% sodium hypochlorite (NaOCl), a mouthrinse containing essential oils and alcohol, and 50% white vinegar.[6] The most effective method for elimination of all tested bacterial species was found to be 50% white vinegar ($p = 0.000$), followed by 2% NaOCl, mouthrinse containing essential oils and alcohol, 0.12% chlorhexidine gluconate, dishwasher use, and tap water (control).[6]

Some studies in rural populations have reported abrasion on the labial surfaces of teeth due to use of charcoal powder for toothbrushing.[15] Although no direct comparison can be made between abrasiveness of charcoal powder and the charcoal-infused toothbrush bristles used in this study, further studies could be done over a longer duration to explore whether charcoal brushes damage the tooth structure. Toothbrush trauma results in portals of entry for micro-organisms, leading to infection.[16] Contaminated toothbrushes can easily be a source of such infections.[16] As a result, various products that claim lesser contamination of used toothbrushes have been developed.[17]

Limitations of the study

One limitation was the lack of analysis of the types of bacteria present. It is possible that anaerobic bacteria may be harboured differently from aerobic bacteria. In future studies, specific types of bacterial growth (aerobic/anaerobic) should be studied. A major study limitation was the lack of an initial power analysis which would have revealed the necessity of using a larger sample size. To compare the effectiveness of the 2 products, studies with a larger sample size will need to be conducted. Additionally, the manufacturers of charcoal toothbrushes have not provided information regarding the concentration of the charcoal in the brush. Thus, the concentration of charcoal at baseline or after a certain period of use cannot be examined with the currently marketed brushes.

Figure 7. Colony counter (Fisher Scientific brand, model F22 0360/10R) used to measure the total colony forming units

CONCLUSION

Our study showed the number of CFUs in charcoal toothbrushes was substantially less when compared with non-charcoal toothbrushes after 1 week of usage. However, the difference in these microbial counts was not statistically significant between the 2 products. Further studies should be conducted with a larger sample size, longer duration of use, and with identification of specific micro-organisms in the bristles.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest in connection with this article.

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