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The Antibacterial Efficacy of Activated Charcoal-Based Toothbrushes on *S. Mutans*

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Abstract

Background: Activated charcoal has been used throughout history as an effective media for the absorption of undesired products. The specific type of charcoal in most charcoal toothbrushes is Binchotan, a white charcoal and several manufacturers claim it possesses antibacterial properties. If found to be effective against *Streptococcus mutans*, these products could be considered as an adjunct to caries prevention treatment for high caries risk patients.

Methods: Brushes selected: Oral-B Charcoal Whitening Therapy Toothbrush, Hello BPA Free Charcoal Bristle Toothbrush and Morihata Binchotan Activated Charcoal Toothbrush. Oral-B 3D was used as a standard nylon control.
Bacterial survival assay: A 0.1 dilution of the *S. mutans* was cultured in sterile saline and one mL was aliquoted to sterile tubes. 25 micrograms of bristles (1/8 of a toothbrush) were added to tubes; additional tubes had nothing added and used as a control. The tubes were mixed for 15 minutes on an orbital rotator at 150rpms. 10 µL aliquots of each tube were spread onto blood agar plates and incubated for 24hrs. After incubation, the number of colonies (CFUs) present on the plates were counted and evaluated. Disk diffusion assay: A 0.5 dilution of the *S. mutans* was cultured in sterile saline and spread evenly across blood agar plates. 25 micrograms (1/8 of a toothbrush) of each bristle type were placed on the plates and incubated for 24 hrs. then observed for zones of inhibition.

Results: Morihata brushes had significantly lower mean CFU counts compared to both Oral-B and non-infused brushes. No pairwise differences were observed between control, Oral-B, and Hello brushes Concentrations of activated charcoal were not high enough to produce a visible zone of inhibition in any of the bristles tested.

Conclusion: Though Morihata charcoal-based toothbrushes demonstrated a significant difference in comparison to the control and other samples, its effect was rather minimal in the patient-centered outcomes of *S. mutans* reduction and caries prevention. Additional *in vivo* and *in vitro* studies of greater sample size are needed before recommending charcoal-based toothbrushes for high caries risk populations.

Introduction

The current landscape of the American consumer is the desire for all-natural and non-toxic products. These trends can be seen in the foods we eat, ingredients we use for cleaning and in beauty and hygiene products. It is only natural that the trend has evolved the dental world as well and it has come in the form of activated charcoal-based toothbrushes and toothpaste.

Activated charcoal, or activated carbon in general, has been used throughout history and throughout the world as an effective media for the absorption of undesired products. It has been proven to be successful in water filtration, an antidote for drug overdoses, reducing cholesterol levels and promoting kidney function. Less evidence-based claims are that activated charcoal helps with acne, flatulence and hangover prevention as well[1]. It is currently being touted as a natural remedy for whitening teeth and promoting a bacteria and odor-free oral cavity. The charcoal interacts with the dentition as the major ingredient in charcoal-based toothpaste or in this study is infused into the bristles of a dental toothbrush[2].

Activated charcoal is a fine black powder made from bone char, coconut shells, peat, petroleum coke, coal, olive pits or sawdust. The charcoal is "activated" by processing it at very high temperatures. The high temperatures change its internal structure, reducing the size of its pores and increasing its surface area. This results in a charcoal that is more porous than regular charcoal. The theory is that when the activated charcoal contacts the tooth surface it absorbs odor and disease-causing bacteria as well as dark colors from coffee, tea, smoking and other stain-producing products[3].

The specific type of charcoal in most charcoal toothbrushes is Binchotan, a white charcoal traditionally used in Japanese cooking. The raw material is oak, specifically ubame oak, with the town of Minabe, Wakayama producing more Binchotan than any other town in Japan[4].

Though these claims have gained the attention of people seeking quick fixes to yellow teeth and bad breath, a scarce amount of research has been conducted on these products as of today. Skepticism exists in the dental community which questions the abrasiveness of charcoal which can lead to enamel damage and tooth sensitivity. Most charcoal toothpastes are absent of fluoride which is proven to aid in remineralization of teeth[5]. As far as charcoal toothbrushes are concerned, how long does the charcoal actually remain activated on a toothbrush? If it is only for one or two uses it would prove to be grossly inefficient and also extremely costly.

Very little statistical research has been found or conducted in the efficacy of charcoal toothbrushes. A 2017 literature review examined prior research regarding charcoal toothpaste and its whitening and antimicrobial claims but found no statistically significant evidence supporting these claims[6]. One study tested the bacterial contamination of charcoal tooth brushes versus non-charcoal toothbrushes. Samples were collected from 90 patients who brushed with both types of toothbrushes and the bristles were placed on agar plates and CFUs were calculated. Non-charcoal bristles contained almost twice as many CFUs than that of the charcoal bristles, however there was no statistically significant difference between the two groups[7].

Another study was able to confirm the antibacterial effectiveness of bamboo charcoal on *Streptococcus mutans* in vitro. Compared to the control sample, a sample exposed to bamboo charcoal powder had a 58.8% antibacterial effect against *S. mutans*. In addition, inhibition zones (circular areas used to measure the susceptibility of bacteria towards an antibiotic) were formed at bamboo charcoal concentrations of 2% and 5%, and 10%[8].

The purpose of this study is to focus primarily on *S. mutans* and confirm the antibacterial effectiveness in vitro of three different charcoal toothbrushes available over the counter on the market today. If one or more of the products prove effective enough to have a bactericidal effect on *S. mutans*, this study can be the springboard for continued research in antibacterial effectiveness of these and similar products in human studies. If antibacterial effects on charcoal toothbrushes on *S. mutans* proves significantly significant in human studies, charcoal toothbrushes may be considered a noteworthy tool for minimizing bacterial load and CFUs present in high-caries risk patients.

Materials and Methods

Three over-the-counter, readily accessible charcoal-infused bristle toothbrushes were analyzed. Brushes selected were as follows: Oral-B Charcoal Whitening Therapy Manual Toothbrush (Proctor and Gamble, Cincinnati, OH), Hello BPA Free Charcoal Bristle Toothbrush (Hello Products, Montclair, NJ) and Morihata Binchotan Activated Charcoal Toothbrush (Morihata International, Philadelphia, PA). A standard nylon toothbrush, Oral-B 3D White (Proctor and Gamble, Cincinnati, OH) was used as a control for comparison of anti-bacterial effects. All samples were tested with a bacterial survival assay and a disk diffusion assay.

For the bacterial survival assay an overnight culture of *Streptococcus mutans* was grown in a tryptic soy broth (TSB) at 35°C. A 0.1 McFarland dilution of the *S. mutans* was cultured in sterile saline and one mL was aliquoted to sterile tubes. 25 micrograms of tooth bristles (approximately 1/8 of a toothbrush) were added to 30 tubes for each of the samples tested as well as 10 tubes had nothing added to be used as an additional control for a total of 130 tubes. The tubes were then incubated at room temperature for 15 minutes on an orbital rotator at 150rpms.10 µL aliquots of each tube were plated onto 5% Sheep Blood Agar (SBA) plates and placed in a 35°C incubator for 24hrs. After incubation, the number of colonies (CFUs) present on the plates were counted using a mobile counting app and evaluated. 2-way ANOVA analysis was used with a significance level was set at $p < 0.05$. Mean values for CFU counts and 95% confidence intervals for the mean were determined for the 5 groups. Antibacterial effect was calculated for each sample group compared to the control average and also compared to each other.

For the disk diffusion assay an overnight culture of *Streptococcus mutans* was grown in TSB at 35°C. A 0.5 McFarland dilution of the *S. mutans* culture in sterile saline was created. The *S. mutans* solution evenly across 30 Mueller Hinton (MH) agar plates for each condition to be tested for a total of 120 plates. 25 micrograms (about 1/8 of a toothbrush) of bristles was placed on the center of the disk for each sample type. The plates incubated overnight in a 35°C incubator.

After incubation, the zone of inhibition around each disk using calipers and the average size of inhibition was compared to the other conditions versus the control.

Data Analysis

Exploratory data analyses were conducted on sample CFUs. The Shapiro-Wilk test was used to assess the normality of the data distribution. Consequently, for continuous data, measures of central tendency are presented as means with associated standard deviations. The non-infused controls and nylon brushes were compared using an independent samples t-test to assess mean differences in CFU count. For subsequent analyses, the nylon and control brushes were pooled as the non-infused group. An analyses of variance (ANOVA) was conducted to check for overall differences in mean CFUs between brush types followed by post-hoc comparisons using Tukey's HSD. Levene's test verified the assumption of homogeneity of variances. An independent samples t-test examined differences between infused vs non-infused brush types. Statistical significance for all statistical tests was declared at $P < 0.05$. Data were analyzed using SPSS 25.0 (IBM, Armonk, NY, USA).

Results

Mean CFU counts for each brush type are shown by group in Table 1. The greatest mean CFU count was observed among the OralB brushes ($n = 30$; $M = 101$, $SD = 22$) while the smallest mean CFU count was found among the Morihata brushes ($n = 30$; $M = 88$, $SD = 14$). An independent samples t- test was conducted to assess differences in mean CFU counts between the two non-infused groups (nylon and controls). No significant difference in CFU count was observed between controls ($M = 103$, $SD = 19$) and nylon ($M=99$, $SD = 21$) brush types thus indicating that the nylon material had no effect on CFUs.

Next, the non-infused and charcoal infused brushes were compared using a one-way ANOVA to compare the effect of charcoal infusion on mean CFU counts. There was a significant effect of brush type on observed CFUs, $P = 0.011$, $\eta 2 = 0.08$. Post hoc comparisons indicated that the Morihata brushes had significantly lower mean CFU counts compared to both OralB and non-infused brushes ($P = 0.045$ and $P = 0.039$ respectively). No pairwise differences were observed between control, OralB, and Hello brushes (all $P >0.05$).

To check whether our non-significant results were due to a lack of statistical power, a post hoc power analysis was conducted using statistical power ($1 - \beta$) set at 0.80 and a two-tailed $\alpha = 0.05$. This demonstrated that given the observed variance in mean CFUs, the total sample size would have to increase to $N = 234$, in order for group differences to reach statistical significance at the 0.05 level. With the available sample of 120, the estimated achieved power given the observed effect size ($d = 0.37$) was0.48. Thus, it is reasonable that the negative findings between infused and non-infused brush types may be attributed to an insufficient sample size.

Table 1. CFU Summary by Group

Brush Type	n	Mean	SD	P[1]
Non-infused				
Control	10	103	19	0.563
Nylon	30	99	21	
Charcoal Infused				
Oral B	30	101	22	
Hello	30	91	16	0.022
Minohata	30	88	14	

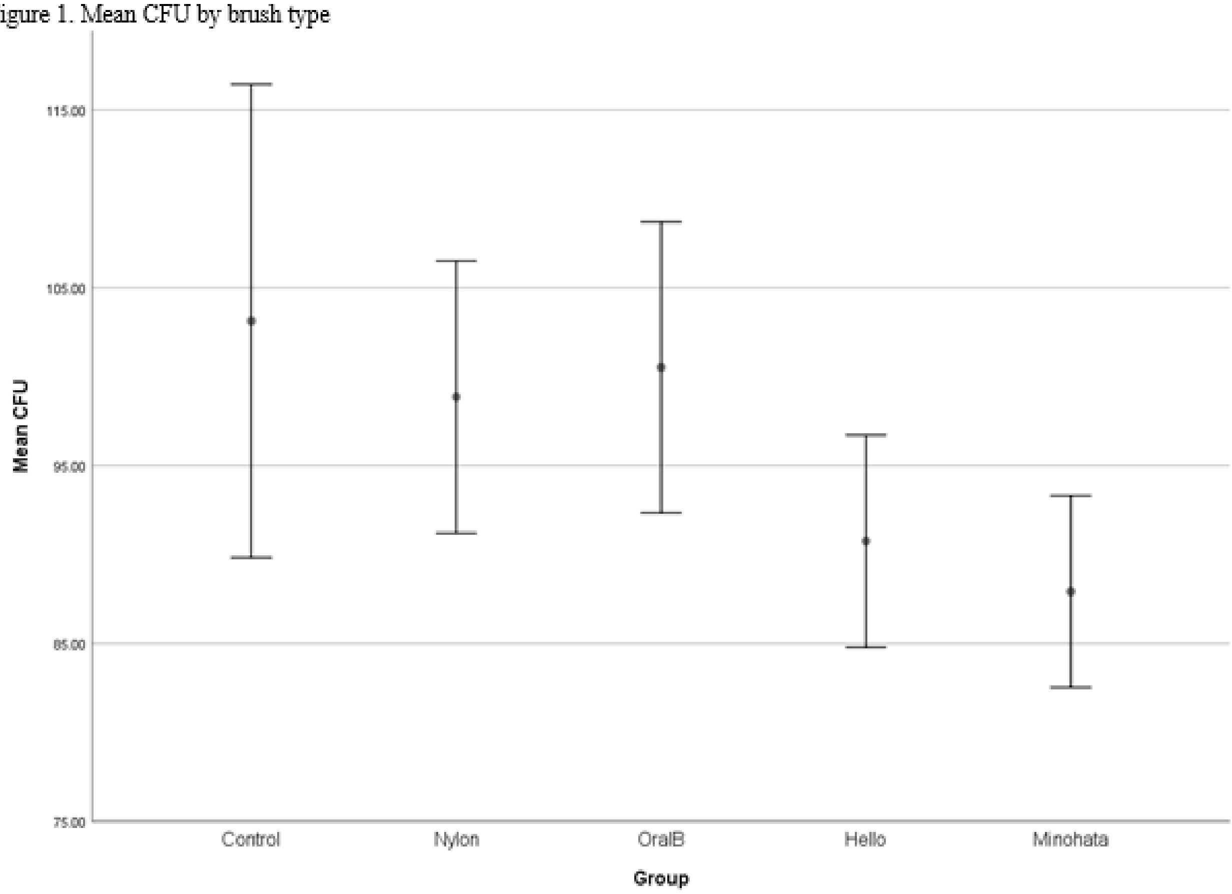
[1] Significance based on intragroup difference in means.

Table 2. Complete CFU results

	Nylon	Oral B	Hello	Minohata	Control
Plate 1	72	107	81	59	136
Plate 2	75	150	81	73	77
Plate 3	107	147	64	87	88
Plate 4	54	81	60	75	102
Plate 5	67	136	57	61	105
Plate 6	92	93	64	78	114
Plate 7	142	115	105	82	108
Plate 8	103	46	100	82	76
Plate 9	79	75	73	106	118
Plate 10	77	113	105	71	107
Plate 11	135	134	95	93	
Plate 12	124	99	101	101	
Plate 13	96	81	82	68	
Plate 14	94	88	102	87	
Plate 15	105	94	98	109	
Plate 16	101	116	97	108	
Plate 17	81	111	97	101	
Plate 18	83	99	98	115	
Plate 19	120	97	92	80	
Plate 20	86	103	101	97	

Plate 21	99	101	117	87	
Plate 22	106	90	99	106	
Plate 23	111	87	87	88	
Plate 24	91	110	101	77	
Plate 25	127	101	115	94	
Plate 26	116	98	110	79	
Plate 27	99	77	76	100	
Plate 28	95	80	98	89	
Plate 29	112	91	78	87	
Plate 30	118	96	89	99	

Figure 1. Mean CFU by brush type



Error bars represent 95% Confidence Intervals

Discussion

The protocol was designed independently by the authors (JCL, MJW). Before implementing the protocol, several variations to this trial were conducted to determine the optimum methodology to attain a quantifiable amount of CFUs on the agar plates. Alterations in mixing time, exposure time, inoculation temperature, bristles per vial and initial concentrations of *S. mutans* were all variables considered. The preparation trials demonstrated that any variations to the values evaluated would generate agar plates with CFU counts so large that they were unable to be accurately calculated by the digital counter or would fail to produce any CFUs at all. The final protocol proposed in this paper was implemented to predictably recreate CFU counts ranging in values less than 200 to be quantifiable data that could be accurately tallied by the digital counter.
Results from this protocol did not mirror the findings of previous studies. Possible causes to the discrepancy of results compared to prior research are that other trials used different brands of brushes and attained their bacterial colonies from *in vivo* clinical trials. They did not test for one specific strain of *S. mutans*, rather the focus was on the overall bacterial load remaining on charcoal brushes versus nylon. Another discrepancy is that previous trials examined the remaining bacteria on brushes after use while this experiment examined the inhibition of growth when charcoal bristles are immersed in a bacterial solution. The findings of this paper suggest charcoal brushes are more effective in maintaining a cleaner, antibacterial toothbrush when not in use.
Statistical analysis demonstrated a significant difference in the CFU count of Morihata bristles versus all other charcoal brands, however the degree of difference when considering minimizing the bacterial load for the consumer is negligible. First, CFUs still formed while exposed to the Morihata bristles which would question the efficacy of minimizing bacterial loads in the oral environment in a high-caries risk patient. Second, the results cannot demonstrate if there would be a lasting effect in the mouth after exposing the bristles to the oral environment while brushing. Though the bacteria may be reduced on the toothbrush itself for a longer duration, it can be assumed the oral cavity would begin to populate *S. mutans* once the exposure ceased. In the trial, the *S. mutans* solutions were exposed to the bristles for 15 minutes while on an orbital rotator, this in theory mimics brushing for 15 minutes. The suggested brushing time for consumers is 2 minutes so it can be concluded that there would be a more minimal effect of the bristles to *S. mutans* when exposed for 2 minutes versus 15 minutes. Finally, Morihata brushes range in price from $8-10 per brush while the Oral-B and Hello brushes range from $2-3 apiece. The Morihata brushes were also coarser in comparison to the Oral-B and Hello brushes. Morihata brushes may have minimal anti-bacterial efficacy over its counterparts, but due to being 3-4 times more expensive and much more abrasive to tooth structure additional research is required before recommending Morihata charcoal toothbrushes over its competitors.
The disk diffusion assays were unable to produce zones of inhibition demonstrating the antimicrobial activity of the charcoal brushes. Explanations for these findings include bristles were used in comparison to charcoal tablets in previous studies. The actual concentration of activated charcoal on the bristles is unknown and can be assumed to be less than the concentration (2% according to previous studies) necessary to produce a zone of inhibition. Higher concentrations would be more costly to the manufacturer as

well as create possible health complications such as gingival inflammation and abrasion if at too high a concentration. Also, it is unknown exactly how long the charcoal on the bristles is “activated” or how many uses until the activation availability is eliminated. The packaged charcoal brushes may lose their activated effectiveness before they reach the consumer. If found to be effective in future studies, additional tests would be needed to examine how many brushing cycles the samples can be used before they lose the effectiveness.

Conclusion

Morihata brushes had significantly lower mean CFU counts compared to both Oral-B and non-infused brushes (P = 0.045 and P = 0.039 respectively.) However, the benefit to the consumer when considering overall anti-bacterial effect is minimal and cannot be recommended over its counterparts. The efficacy of charcoal brushes and the ability to create zones of inhibition were not able to be reproduced from previous studies. The efficacy of charcoal-infused brushes in their capacity for purchase in the public market do not appear to be antibacterial simply by introducing them to an environment containing *S. mutans*, rather are an additive to maintain a more bacteria-free toothbrush when not in use. Consumers may still desire to utilize charcoal-based toothbrushes for their potential to brighten teeth and debride plaque, but further studies are needed to confirm the antibacterial efficacy of these products in the oral environment. Guidance for the management of high-caries patients remain to include scientifically-backed concepts of proficient oral hygiene and maintenance, use of antimicrobial agents such as chlorhexidine and nutritional counseling.

References:

1. Hill, A. What Is Activated Charcoal Good For? Benefits and Uses https://www.healthline.com/nutrition/activated-charcoal

2. Wheeler, G. Charcoal toothbrushes: what are the benefits and which is the best one? https://www.electricteeth.co.uk/charcoal-toothbrush/

3. Konno H1, Yaegaki K, Tanaka T, Sato T, Itai K, Imai T, Murata T, Herai M. Neither hollow-fibre membrane filters nor activated-charcoal filters remove fluoride from fluoridated tap water. J Can Dent Assoc. 2008 Jun; 74(5):443.

4. Benefits of Binchotan Charcoal Toothbrushes https://www.nourishedlife.com.au

5. Mosquim V, Martines Souza B, Foratori GA, Junior, Wang L, Magalhães AC. The abrasive effect of commercial whitening toothpastes on eroded enamel. Am J Dent. 2017;30(3):142–146.

6. Brooks JK, Bashirelahi N, Reynolds MA. Charcoal and charcoal-based dentifrices: A literature review. J Am Dent Assoc. 2017 Sep;148(9):661-670.

7. Lee, J et al. Comparison of bacterial contamination in bristles of charcoal toothbrushes versus non-charcoal toothbrushes. Canadian Journal of Dental Hygiene. Jun 2013, Vol 51:69-75.

8. Choi, MS, Ahn KS, Antibacterial Effect of Bamboo Charcoal on *S. Mutans*. Journal of Korean Society of Dental Hygiene. 2014 Feb 14:95-100.

9. Lawal FB, Taiwo JO, Oke GA, Oral Health Practices of Adult Inhabitants of a Traditional Community in Ibadan, Nigeria. Nigerian Journal of Medicine. Nov 2013, 22:212-7.

10. Kaur, A et al. Comparative Assessment of the Efficacy of two commercially available Manual Tooth Brushes for removal of Dental Plaque. J of Ad Medical and Dental Sciences Research. 2018 Jun: 62-65