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"The role of a toothbrush in tooth brushing, intra-oral bacteria, halitosis and its general systemic health implications"

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Simple tooth brushing can reduce oral bacteria by 95%. Effective toothbrushing represents a simple but beneficial method for influencing intra-oral bacterial levels, which can dramatically impact health and drive down the costs at many levels of oral and general health problems.

INTRODUCTION

Recently, scientific literature has been linking intra-oral bacteria to a number of systemic diseases [1,2,3], including cardiovascular disease [4,5] stroke [37,38,39,40,41], preterm birth [9], diabetes [18], and respiratory diseases [6,7,8]. Tooth brushing can reduce, control and/or maintain a better intra-oral bacterial environment, potentially improving self-induced, chronic disease states, such as periodontal disease. Periodontal disease accounts for inordinate intra-oral bacterial activity has found itself intimately involved with the above somatic disease states. These somatic disease states account for a great majority of health care treatment and costs.

Any improvement in physical health is in itself beneficial, but there are other benefits. With improved health an individual spends less time with the physician and hospital, thereby saving thousands and potentially millions of dollars. The financial savings not only mean less medical costs but also savings made with an improved work force.

When an individual is not doing their job, then the product/service is not being delivered. Often times, it takes another individual to make up for that medically compromised worker. Often times, the remaining healthy individuals will be tasked to make up for that dental compromised individual that can not be at work.

Therefore, if an individual can better maintain their intra-oral bacteria while using a good, simple toothbrush, then the quality of life can be improved with a stronger work force, financial savings and noticeably improved individual social skills.

In recent years, it has been scientifically substantiated that intra-oral bacteria can cause or contribute to general somatic health problems involving the heart, the kidneys, liver, pregnancies and respiratory disorders [1,3,5,6,7,8, 9,10]. Chronic poor oral hygiene that contributes to an inordinate amount of intra-oral bacteria can then contribute to any of the above somatic health problems and will become a serious issue. The physical toll on the patient is but the enormous financial toll is often overlooked in comparison to the solution with an good toothbrush. Treating general somatic diseases can cost thousands, if not millions, of dollars. The years of cost to treat a myocardial infarction, heart attack patients can be several hundreds of thousands of dollars with a questionable outcome [42,43]. The cost of an average OTC toothbrush can range from $2.00 to $20.00 [11] with a very predictable positive outcome. The positive of aspects of a good toothbrush and oral hygiene is self-evident.

The benefits of improved tooth brushing and oral hygiene can be easily observed by the dentist both visibly and other diagnostic techniques. That is good for the dentist who will see the patient every six months but what technique is available for the patient? The patient would benefit from some type of positive reinforcement that is easy to observe. Generally this would be through objective means, ie, the senses, visual and olfactory. What the patient can see and smell are enormous positive reinforcement techniques.

The implications of improved oral hygiene, along with a simple technique for a patient to observe and reinforce oral hygiene improvements can improve chronic health issues and ultimately general somatic health. This, in turn, can save significant financial assets and productive work hours that can be better utilized in other budgeted areas.

The aim of this study is to observe the effects of regular tooth brushing on the intra-oral bacterial environment. Intra-oral bacterial samples were taken from the tongue prior to tooth brushing (pre-brush) and after tooth brushing (post-brush) utilizing a sterile tongue swab. Tooth brushing was accomplished without toothpaste, rinsing with regular sink water from a faucet. The oral areas brushed included the teeth, surrounding soft tissue, palate and the tongue. The Modified Bass method [12] tooth brushing technique was utilized taking approximately 3 minutes to perform.

Identification of bacterial types and counts were observed in simple, real scenario, pre-brush and post-brush analysis. This was accomplished using culture techniques, PCR products followed with DNA sequencing.

MATERIALS AND METHODS

Intra-oral Sampling

This simplified study utilized a healthy individual with an uneventful health history with no prescriptive or OTC drug history. Normal eating habits were maintained with breakfast, lunch, dinner and occasional snacks.

Multiple bacterial samples were taken consistently at the same time and same intra-oral location. Sample collections were taken at noon after lunch. Normal tooth brushing was maintained after breakfast, lunch, dinner, and prior to going to sleep. The noon brushing was utilized during the sampling for this study.

Each tooth brushing sampling utilized a ***new toothbrush that is commercially available [44]***. Each toothbrush had been pre-sterilized (ethylene oxide treatment). Pre-brush and post-brush intra-oral bacterial samples were taken from the tongue dorsum utilizing the sterile tongue swab. The tongue swabbing technique used was accomplished by rotating the swab head and then by swabbing the head side to side for one minute over the entire tongue dorsum area. Tooth brushing was accomplished with no toothpaste and only rinsing with regular sink water. The intra-oral areas brushed included the teeth, surrounding soft tissue, palate and the tongue. The Modified Bass method [12] tooth brushing technique was utilized taking approximately 3 minutes to perform.

Bacterial Culture Methods

Pre and post-brushing sample swabs were washed in 1ml 1x PBS. The inoculated PBS was serially diluted and plated onto three types of agar; Luria Bertani (LB), Todd Hewitt (THA; Becton-Dickinson), and Trypticase Soy (TSA; Becton-Dickinson).

Colony counts were obtained from these plates and used to determine total colony forming units per ml, or per sample. The % difference of bacteria from pre and post-brushing samples was then determined.

The same sample swabs were then used to streak swab onto LB, THA and TSA plates to visualize bacterial numbers in an undiluted sample and for isolation of different colony types. All plates were incubated overnight at 37° in 5% CO2.

Chromosomal Extraction

Unknowns were grown overnight at 37° in a shaker incubator. Gram staining was performed prior to extracting DNA for each colony type. Genomic extraction for gram-negative bacteria was done using DNeasy Blood & Tissue Kit (Qiagen Sciences, Maryland). Gram-positive genomic DNA extraction was performed using Wizard Genomic DNA Purification Kit (Promega Corp, Madison, WI).

16S rRNA Gene Amplification and Sequencing

Identification of bacterial species was done by PCR amplification of the 16S rRNA gene to obtain DNA sequencing data for each colony type. Gene amplification was carried out using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR conditions included a preheat step of 95°C for 5 min, followed by thirty cycles of amplification with the following conditions: denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and elongation at 68°C for 2 min, followed by a final elongation step at 72°C for 15 minutes.

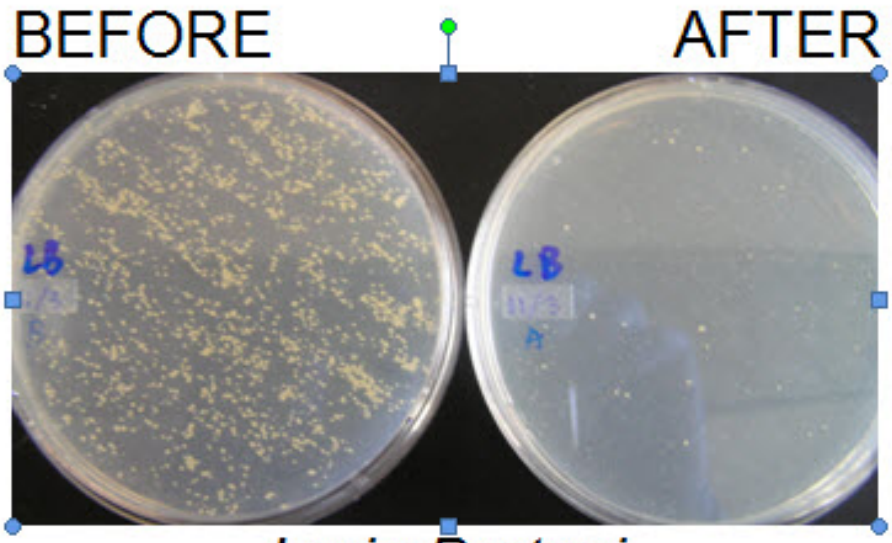
x Fraction of PCR reaction was electrophoresed on a 1% Agarose gel for 1.5 hrs at 100V to verify presence of 16S rRNA gene and the specificity of the PCR. PCR products were then purified using Wizard SV Gel and PCR Clean-Up System (Promega Corp, Madison, WI) and sent out for sequencing using the same primers to SDSU Microchemical Core Facility (San Diego, CA).

DNA sequencing data was analyzed using the BLASTN database of NCBI (http://www.ncbi.nlm.nih.gov) [35].

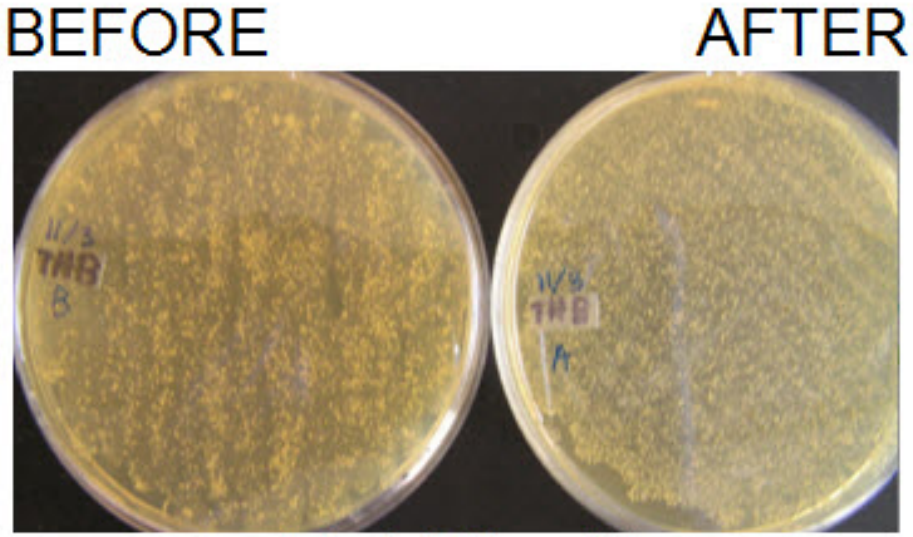
RESULTS

A total of 12 sets of data that included pre and post-tooth brushing were obtained over 4 days. Great care was taken to maintain consistency in this study by keeping a similar diet, timing and method for all samples used, mimicking an average tooth brushing individual. Each trio of media is representative of one sample in order to appreciate any variability in species selection between media.

Even while samples plated using the streak swab technique gave lawns of bacteria, an overall decrease could be seen when comparing pre and post-tooth brushing plates (Fig 1).



Luria Bertani



Todd Hewitt

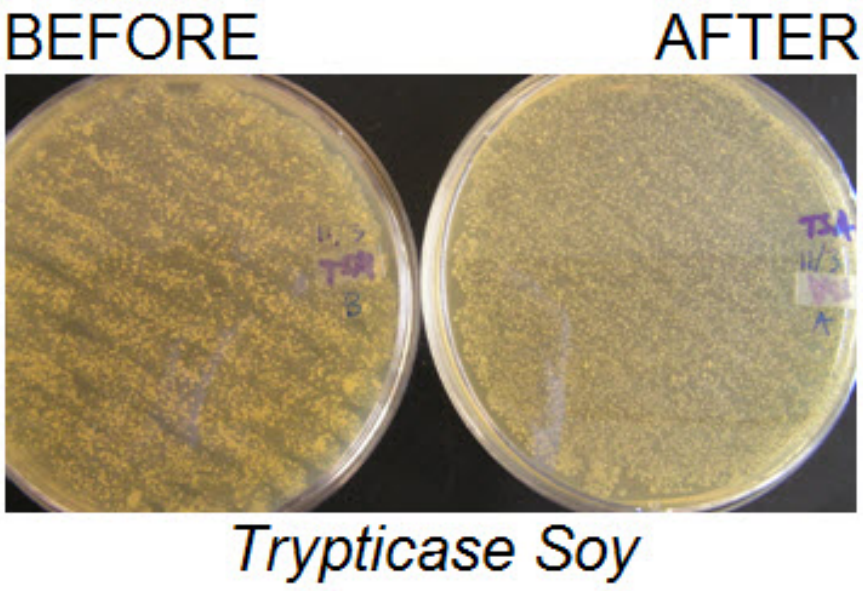


Figure 1. Decrease in bacterial abundance after brushing is evident on all plates.

Quantitative data was obtained from colony counts on the dilution plates. As expected, most of the plates showed a ***decrease of 85 to 99%*** when comparing pre and post- brushing cultures (with the exception of two sets that showed a 60 and 68% decrease). The average decrease in bacterial colonies, when including all samples, was of 88.8% (Figure 3). The decrease in bacterial numbers was not selective for a media type; THA, TSA and LB showed an average decrease of 87.3%, 92.8%, and 86.2% respectively (Figure 2).

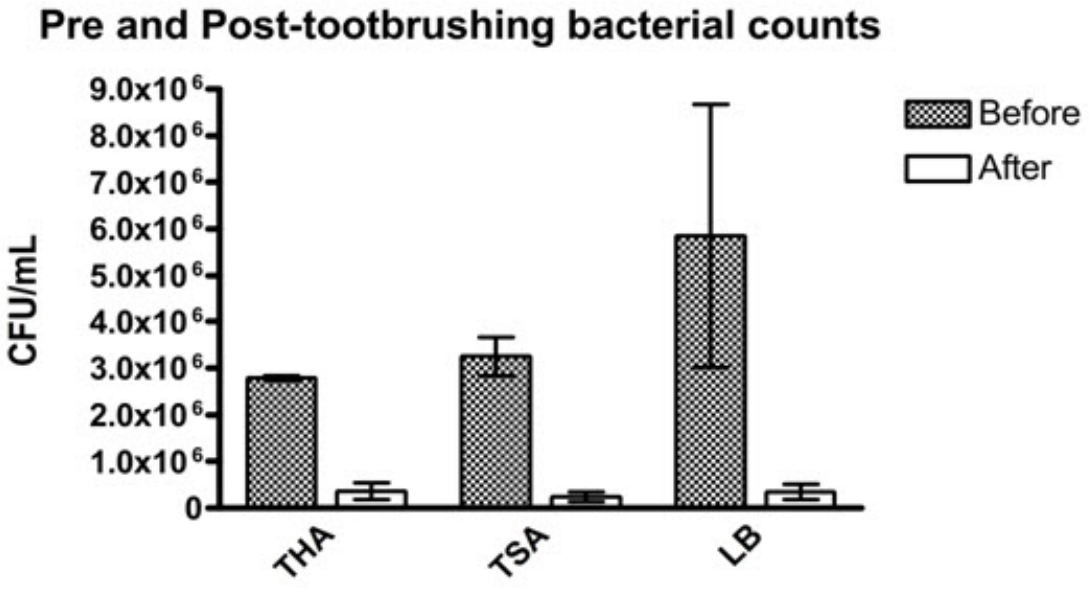


Figure 2. Efficacy of bacterial decrease. All media types show a significant decline in colonies counted.

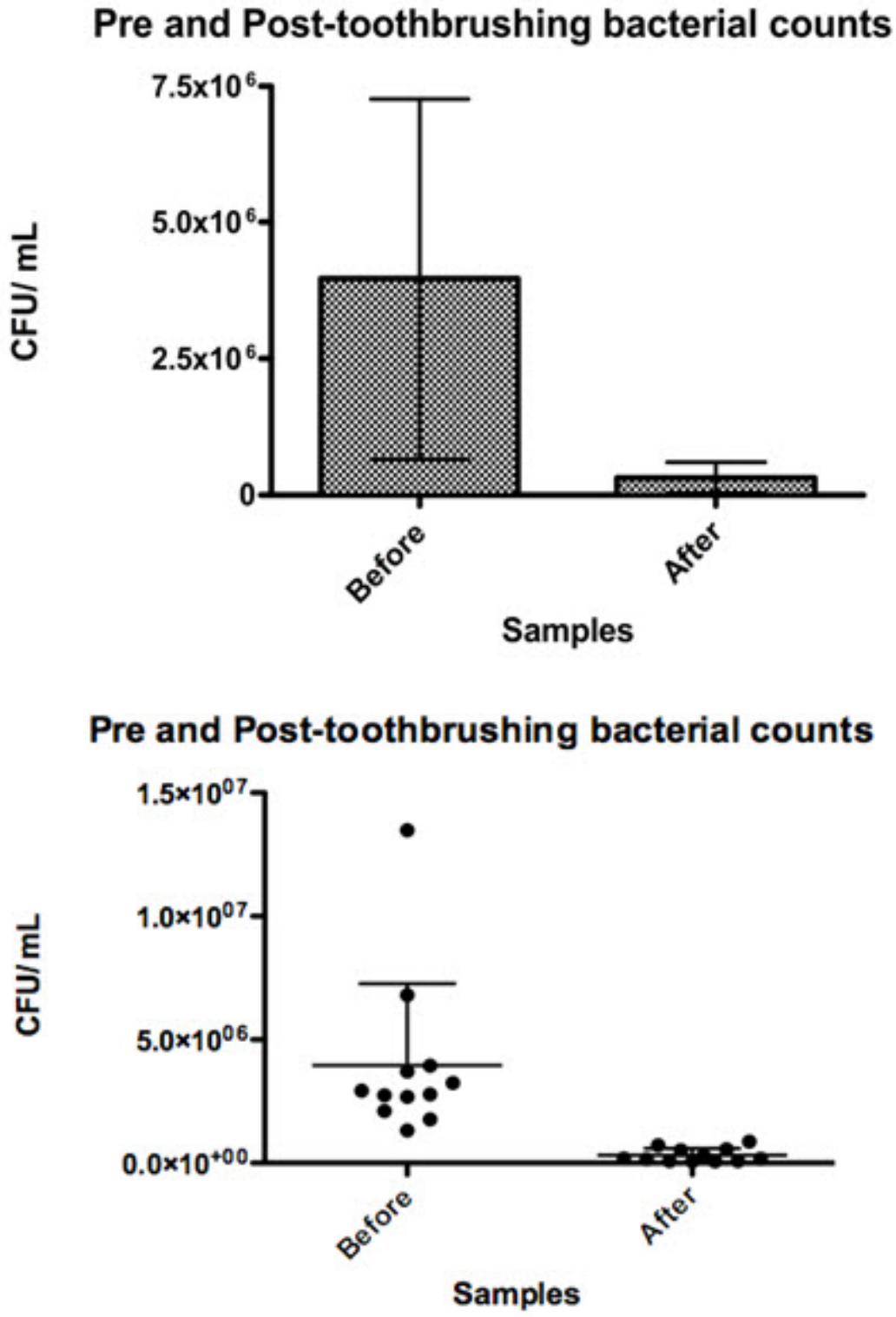
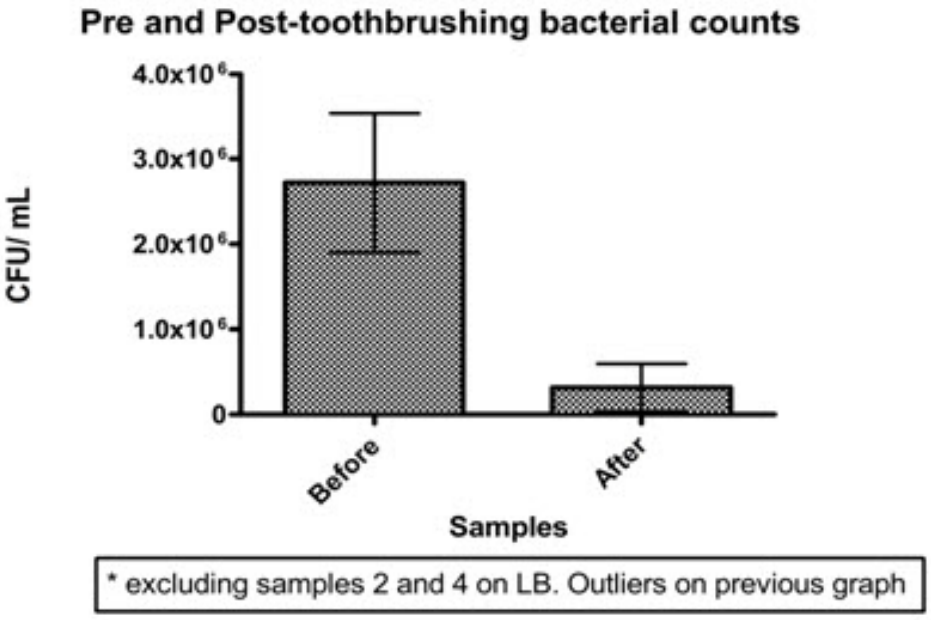


Figure 3. (linear bar & dot plot) Average of twelve samples taken over four days.



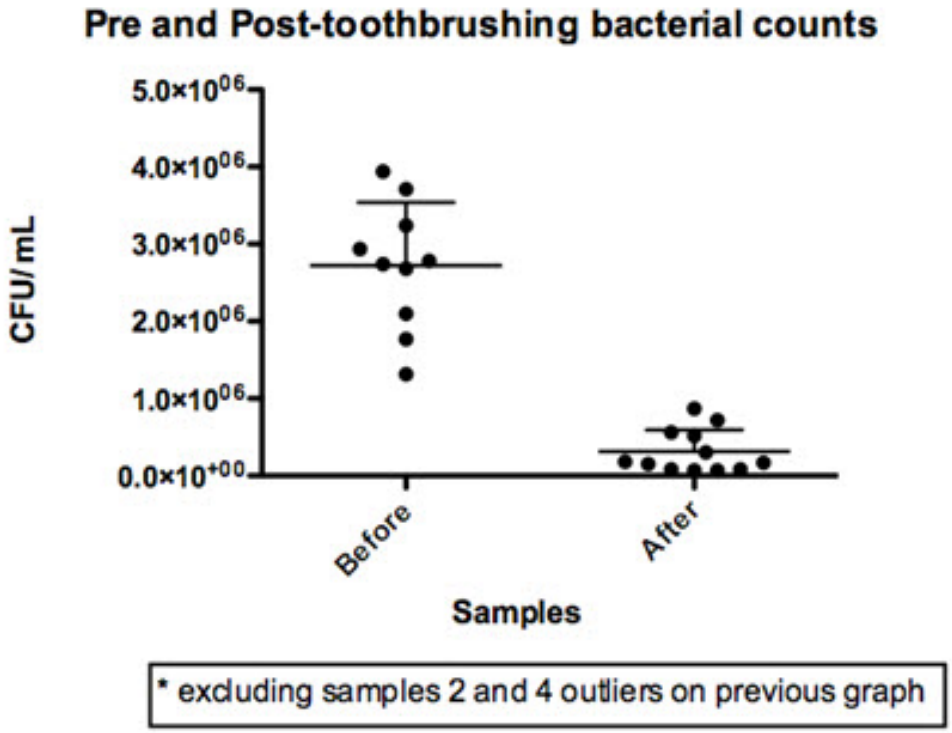


Figure 4. (linear bar & dot plot)Average of ten samples taken over four days.

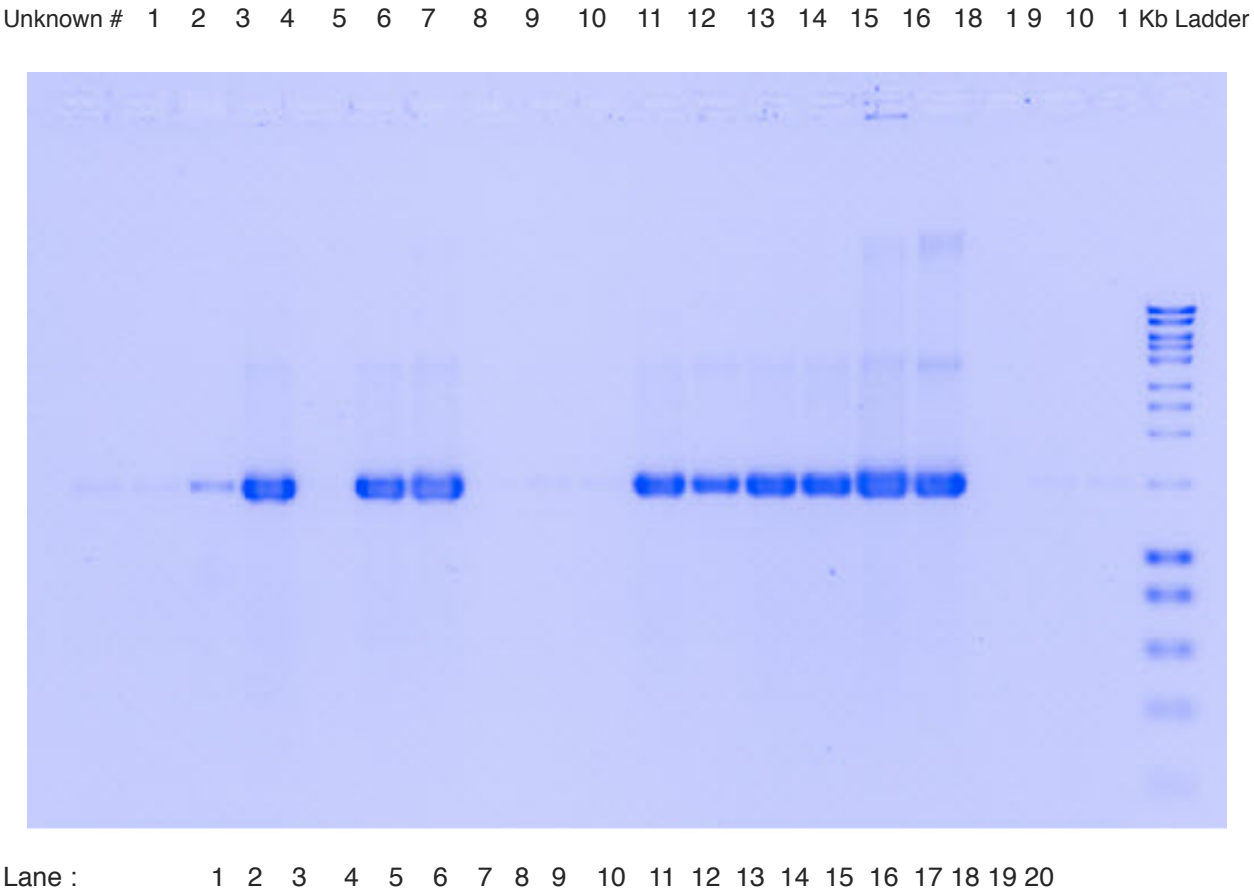


Figure 5. Verification of 16S rRNA was performed using 1% Agarose gel. 5µl of DNA combined with 1 µl of 6X loading dye were loaded onto each well.

Potentially different strains were isolated from streak-swab and dilution plates based on morphology. Their respective morphologies are listed on Table 1. Unknowns 3, 8, 9 and 10 did not give dense overnight cultures and resulted in a small yield of DNA. When these unknowns were used in PCR, their DNA was only minimally amplified, as can be seen by the faint bands on the Agarose (Figure 3).

Unknowns 1, 2, and 5 on the other hand gave dense cultures when grown overnight and chromosomal extraction gave a visible pellet, but also failed to give enough amplification upon PCR. Two PCR reactions were run on DNA from samples 8, 9, and 10 in order to obtain sufficient DNA for sequencing. Samples 8 and 5, which did not give a visible 16s rRNA product band on the gel, were not included in the sequencing part of this study. Remaining PCR tubes were all purified before Duplicates for samples 9 and 10 were combined before they were purified for sequencing.

Analysis of sequencing data showed that the prevalent species in these samples included Nisseria subflava, Staphylococcus aureus, Streptococcus salivarius, Neisseria sp., Uncultured Streptococcus sp., Streptococcus vestibularis, Streptococcus genomosp., Staphylococcus warneri, Acinetobacter sp. (Table 1).

Unknown	Morphlogy	Gram Stain	Sequencing analysis (NCBI)	Max e value	ident
1	frosty-white	+	no results		
2	frosty-white	+	no results		
3	yellow, small	-	Nisseria subflava	6E-131	97%
4	whiteish-yellow	+	Staphylococcus aureus	0	87% *
5	frosty-white, large	+	no results		*
6	frosty, brownish, large	+	Streptococcus salivarius	8E-166	82%
7	orange-yellow, large	+	Staphylococcus aureus	0	97%
8	light brown, glossy, large	+	no results		
9	light brown, glossy, white center	+	no results		
10	light brown, rough edges	+	no results		
11	light brown, small, cubic shine	-	Streptococcus salivarius	0	95%
12	yellow, target look	-	Neisseria sp.	0	96%
13	white, small	-	Uncultured Streptococcus sp.	1E-163	83%
		2nd hit	Streptococcus vestibularis	7E-161	82%
14	translucent, small	+	Streptococcus genomosp.	0	91%
15	yellow, smooth	+	Staphylococcus warneri	0	96%
16	white, smooth	+	Acinetobacter sp.	0	95%

Table 1. DNA sequencing analysis.

Within these bacteria several can cause significant health issues from Neisseria subflava influencing opportunistic infections, such as meningitis, septicemia, and endocarditis [55], with acute bacterial endocarditis caused by Staphylococcus aureus [56], systemic bacteremias caused by Staphylococcus warneri [57] and Acinetobacter sp as a significant antibiotic resistance pathogen involved in pneumonia patients with cystic fibrosis, neutropenia, advanced AIDS, and bronchiectasis. [58].

DISCUSSION

This study indicates that bacteria are ever present in the intra-oral environment utilizing the tongue dorsum as the reference site and how a good toothbrush helps reduce and control intra-oral bacteria. The intra-oral environment (entire mouth cavity) includes the teeth, the palate, surrounding soft tissue and tongue.

The average decrease in bacterial colonies as a result of brushing was an 88.78% decrease, indicating a proper toothbrush can effectively lower potential disease and halitosis causing bacteria.

Even though water was used in this study to rinse the mouth after brushing, it is our belief that water alone, without the toothbrush, would not play a large role in bacterial reduction and that the data presented here is representative of the benefits of a proper toothbrush.

It is estimated that over 100 different types of bacteria are present in the mouth [13,14] making up a total of several billion present [15]. Several of these intra-oral bacteria types have been isolated as a potential causative/contributory factor in cases of myocardial infarctions, respiratory disorders, liver & kidney illnesses and pregnancy complications [1,3,4,5,6,7,8,9,10].

Normal intra-oral bacterial flora [54] is necessary for microscopic homeostasis [16] but it can become a health issue when these bacteria levels become excessively present allowing for a broader bacterial spectrum. It is within this broad spectrum of bacteria where some of the more aggressive bacteria types can create the above health issues.

One of the most basic characteristics of a disease process is a malodor [17, 47]. Malodors or fetid odors can be found in cases of infections (periodontal, halitosis/bad breath, gangrenous, respiratory) [16,19,20,21] and liver/kidney disorders [18,25].

Therefore, anytime an individual can sense a reduction and/or have no oral malodor issues then there is a greater assumption of improving health. Malodors from intra-oral bacteria is a long-standing and well known social problem called “bad breath” or “halitosis” [22, 23, 24, 26].

Halitosis can be utilized as one potential reference of excessive intra-oral bacterial activity [26, 27]. Studies have indicated that the cause for halitosis is due to gram-negative bacteria [46,48], but other studies also indicate that halitosis is caused by gram-positive bacteria [22,45].

Therefore, an easy, field halitosis detection technique has beneficial effects to help reinforce the tooth brushing individual's motivation for a healthy oral environment with a good toothbrush.

One easy technique to detect bad breath is to lick the back of your hand, allow the saliva to dry and then smell [28,29]. If this area does not pass the "smell test" demonstrated with a bad odor, then one aspect of an unhealthy mouth can be assumed. A strong mouth malodor is indicative that excessive, deleterious bacterial activity is present [26,27]. This easy, field technique can help assist an individual as to how well they are doing in their oral hygiene tooth brushing technique.

The end result of reduced intra-oral bacteria will not only improve the quality of life with better breath and teeth but also potentially reduce general somatic health problems. An improved physical quality of life can also be interpreted as an improvement of financial conditions related to health care. A good toothbrush can cost less than $20. The average treatment cost of a myocardial infarction can be over several hundred thousand dollars [30,31]. The cost of a complicated pregnancy and compromised child can be a lifetime of hardships and potentially millions of dollars [33,34]. Ignoring such facts can result in great personal and societal loss.

CONCLUSION

In today's economic and financial climate cost savings are critical. If a good toothbrush can improve oral hygiene potentially saving $100,000 or more, then this toothbrush should be pivotal part of every individual's daily regiment.

The World Health Organization estimates that chronic, self-induced diseases such as 80% of heart disease, type 2 diabetes and more than 40% of cancer could be prevented if Americans stopped smoking, ate healthier and exercised more [49]. Chronic diseases are the #1 cause of death and disability in the United States [50]. Treating chronic disease accounts for 75% of the nation's health care costs [51] and accounts for two-thirds of health care cost increases [53]. When the potential for intra-oral bacteria causing or adding to these chronic diseases exists and when a good toothbrush can reduce these disease states, then this simple cost-benefit toothbrush should be a part of all health care planning.

When these chronic diseases disable a working individual then society, as a whole, is greatly affected. Typically a health compromised individual will not only take them away from their mission but they will also take several others away from their job/mission to support that lost unhealthy individual, both directly and indirectly. The loss of the unhealthy individual from the workforce leaves a personnel "vacuum" that has to be filled by one or more individuals. Aside from the personal loss, enormous amounts of money are also lost that take away from every level of the socio-economic scale.

Chronic disease, health issues reduce economic productivity by contributing to increased absenteeism and poor performance. A Milken Institute Study [52] determined that treatment costs of the seven most common chronic diseases, along with productivity losses, cost the U.S. economy in excess of $1 trillion dollars annually. The same study estimates that just modest reductions in unhealthy behavior patterns could prevent or slow 40 million cases of chronic illness per year. Utilizing a good toothbrush is one simple improvement in healthy behavior.

A hospital can easily be treating a dozen or more patients with serious somatic diseases that were chronically, self-induced. It would not be unusual for the medical costs to then reach in excess of a million dollars per patient. Duplicate this scenario, patient after patient, hospital after hospital, year after year and tens of millions of dollars could easily be spent on these self-induced health compromised patients. If these conditions could be intercepted, even by a mere fraction, before they became a health issue then these millions of dollars saved could be diverted back to the economy and other services.

This simple, cost effective toothbrush, can potentially avoid greater somatic health problems by removing or reducing host intra-oral bacteria that can cause cardiac, respiratory, brain, liver and kidney problems. When almost 90% reductions of intra-oral, malodor, bacteria occur with the toothbrush then its regular use should be a part of everyone's daily health care. The potential savings that are in the millions of dollars are self-evident, especially in light of such enormous "toothbrush vs illness" cost-to-benefit savings ratio.

Additional study and analysis of this subject should be encouraged.

REFERENCES:

[1] Jeffrey Astroth, DDS, MSPH , The links between oral and systemic health The Clinical Advisor, March 2009

[2] Rishirai B, Epstien JB, Basal cell carcinoma; what dentists need to know. J Am dent Assoc. 1999; 130: 375-380

[3] Joshipura KJ, Rimm EB, Douglass CW, et al. Poor oral health and coronary heart disease. J dent Res. 1996;76:1631-1636

[4] Genco R, Chadda S, Grossi S. Periodontal disease is a predictor of cardiovascular disease in a native American population (abstract). J Dent Res. 1997;76:408.

[5] Beck JD, Garcia R, Heiss G, et al. Periodontal disease and cardiovascular disease. J Periodontol. 1996;67(10 Suppl):1123-1137.

[6] Scannapieco FA, Papandonatos GD, Dunford RG. Associations between oral conditions and respiratory disease in a national sample survey population. Ann Periodontol. 1998;3:251-256.

[7] Hayes C, Sparrow D, Cohen M, et al. The association between alveolar bone loss and pulmonary function: the VA Dental Longitudinal Study. Ann Periodontol. 1998;3:257-261.

[8] Yoneyama T, Yoshida M, Ohrui T, et al. Oral care reduces pneumonia in older patients in nursing homes. J Am Geriatr Soc. 2002;50:430-433.

[9] J. H. Meurman, J. Furuholm, R. Kaaja, H. Rintamäki and U. Tikkanen. Oral health in women with pregnancy and delivery complications. CLINICAL ORAL INVESTIGATIONS. Volume 10, Number 2, 96-101,DOI: 10.1007/s00784-006-0037-4

[10] Bayraktar G, Kurtulus I, Kazancioglu R, Bayramgurler I, Cintan S, Bural C, Bozfakioglu S, Issever H, Yildiz A. Oral health and inflammation in patients with end-stage renal failure. Perit Dial Int. 2009 Jul-Aug;29(4):472-9.

[11] Maremont, Mark. New Toothbrush is a big ticket item. The Wall Street Journal Western Edition 1998. ISSN: 0193-2241

[12] Karen Kaiser, RDH. The driving Force. RDH Magazine, Aug 2003, Article/184868

[13] Jørn A. Aas, Bruce J. Paster, Lauren N. Stokes, Ingar Olsen, and Floyd E. Dewhirst..Defining the Normal Bacterial Flora of the Oral Cavity. J Clin Microbiol. 2005. November; 43(11): 5721–5732.

[14] Paul E. Kolenbrander, Roxanna N. Andersen, David S. Blehert, Paul G. Egland, Jamie S. Foster, and Robert J. Palmer, Jr. Communication among Oral Bacteria. Microbiol Mol Biol Rev. 2002 September; 66(3): 486–505.

[15] Bill Landers. Oral bacteria: How many? How fast? RDH Magazine. Volume 29; 7. July 2009

[16] Egija Zaura , Bart JF Keijser , Susan M Huse and Wim Crielaard Defining the healthy "core microbiome" of oral microbial communities.BMC Microbiology 2009, 9:259

[17] Hung-Chih Chang, Laszlo B. Kish, Maria D. King, Chiman Kwan Identification of Bacteria by Patterns Generated from Odor Spectra. arXiv:0901.3100v2 [v1] Mon, 9 Feb 2009 05:25:19 GMT (484kb)

[18] Kim Whitman RN, CCRN, MSN ,Crystal McCormick RN, CCTN., When your patient is in liver failure. Nursing2005. April 2005, Volume 35 Number 4, Pages 58 - 63

[19] Masanori Niimi, MD, Yosifumi Ikeda, MD, Shigenao Kan, MD, Hiroshi Takami, MD. Gas Gangrene in Patient With Atherosclerosis Obliterans. Asian Cardiovasc Thorac Ann 2002;10:178-180

[20] C. E. Kazor, P. M. Mitchell, A. M. Lee, L. N. Stokes, W. J. Loesche, F. E. Dewhirst, and B. J. Paster. Diversity of Bacterial Populations on the Tongue Dorsa of Patients with Halitosis and Healthy Patients. J Clin Microbiol. 2003 Feb; 41(2): 558–563.

[21] Celine Mascaux; Nir Peled; Kavita Garg; Yasufumi Kato; Murry W Wynes; Fred R Hirsch. Early Detection and Screening of Lung Cancer. Expert Review of Molecular Diagnostics. Expert Rev Mol Diagn. 2010;10(6):799-815.

[22] Rosenberg M PhD. Current Concepts in Clinical Assessment of Bad Breath. Am Dent Assoc, 1996 Apr, 127:4, 475-82

[23] Rosenberg M PhD et al., Self-estimation of oral malodor. J Dent Res, 1995 Sep, 74:9, 1577-82

[24] Bosy A: J: Oral malodor: philosophical and practical aspects. Can Dent Assoc, 1997 Mar, 63:3, 196-201.

[25] Michael L. Simenhoff, M.D., James F. Burke, M.D., Jussi J. Saukkonen, M.D., Anacleto T. Ordinario, M.D., Richard Doty, Ph.D., and Stephen Dunn, B.S. Biochemical Profile of Uremic Breath. N Engl J Med 1977; 297:132-135

[26] Ilana Eli, D.M.D., Roni Baht, Ph.D., Hilit Koriat, D.M.D. and Mel Rosenberg, Ph.D. Self-perception of breath odor. J Am Dent Assoc, Vol 132, No 5, 621-626.

[27] Violet I. Haraszthy, DDS, MS, PhD, Joseph J. Zambon, DDS, PhD, Prem K. Sreenivasan, PhD, Margaret M. Zambon, MPH, Doralee Gerber, BS, Rodrigo Rego, DDS and Carol Parker, BS. Identification of oral bacterial species associated with halitosis. J Am Dent Assoc, Vol 138, No 8, 1113-1120.

[28] Mel Rosenberg Ph.D.1 . Clinical Assessment of bad Breath: Current Concepts. J Am Dent Assoc, Vol 127, No 5, 570

[29] Tomas O'Caomhanach. The Bad Breath Report. http://www.thebadbreathreport.com/bad-breath-test.html

[30] Steve Vernon . How Much Would a Heart Attack Cost You?. CBS Money Watch. Apr 23, 2010. http://moneywatch.bnet.com/retirement-planning/blog/money-life/ how-much-would-a-heart-attack-cost-you/799/

[31] The WISE Study. Shaw LJ, Merz CNB, Pepine CJ, et al. “The Economic Burden of Angina in Women With Suspected Ischemic Heart Disease: Results From the National Institutes of Health-National Heart, Lung, and Blood Institute-Sponsored Women’s Ischemia Syndrome Evaluation.” August 29, 2006 2006;114(9):894-904.

[32] Steven Jaksha, CDR, DC, USNR. It's Only a Dental Exam. NRA News. Health Affairs. Sept 2004: 23.

[33] Roger J. Stancliffe, K. Charlie Lakin, John R. Shea, Robert W. Prouty, and Kathryn Coucouvanis. Excerpts from The Economics of Deinstitutionalization1; 2

[34] A Honeycutt, PhD, L Dunlap, MA, H Chen, MA, G al Homsi, MA, RTI International, Research Triangle Park, North Carolina. S Grosse, PhD, D Schendel, PhD, Economic Costs Associated with Mental Retardation, Cerebral Palsy, Hearing Loss, and Vision Impairment. USA, 2003 National Center on Birth Defects and Developmental Disabilities, CDC. 1/30/2004 / 53(03);57-59

[35] National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/

[36] Hillman JD, Socransky SS. The theory and application of bacterial interference to oral diseases. In New Biotechnology in Oral Research. Ed. Myers HM (Basel: S Karger) 1989; 1-17

[37] Pulsinelli W. Excitotoxic damage in global ischemia. Adv Neurol. 1996;71:61-62.

[38] Syrjanen J, Peltola J, Valtonen V, et al. Dental infections in association with cerebral infarction in young and middle-aged men. J Intern Med. 1989;225:179-184.

[39] Grau AJ, Buggle F, Heindl S, et al. Recent infection as a risk factor for cerebrovascular ischemia. Stroke. 1995;26:373-379.

[40] Joshipura KJ, Hung HC, Rimm EB, et al. Periodontal disease, tooth loss, and incidence of ischemic stroke. Stroke. 2003;34:47-52.

[41] Janket SJ, Baird AE, Chuang SK, et al. Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;95:559-569.

[42] Vernon, S.. "How Much Would a Heart Attack Cost You"?. CBS Money Watch. Apr. 23,2010

[43] Brown, D. “A Case of Getting What You Pay For”. Washington Post. 7/26/2009.

[44] Jaksha, S. DMD, NeutralizerPLUS+: Changes in Toothbrush Technology, DENTAL INNOVATIONS; Mar 2010

[45] Sterer, N. et al. b-Galactosidase Activity in Saliva is Associated with Oral Malodor. J. Dent. Res. (2002) 81: 182

[46] Bosy A, Oral malodor: philosophical and practical aspects. J Can Dent Assoc. 1997 Mar; 63(3):196–201

[47] Tangerman A. Halitosis in medicine: a review. Int Dent J. 2002Jun;52 Suppl 3:201-6.

[48] Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. J Periodontol. 1977 Jan;48(1):13–20.

[49] Preventing chronic Diseases: A Vital investment: WHO Global Report. 1. Chronic Disease-Therapy 2. Investments 3. Evidence-Based medicine 4.public Policy 5. Intersection Cooperation I Title. ISBN 92-4-156300-1

[50] Centers for Disease Control and Prevention. Chronic Diseases and Health Promotion. http://www.cdc.gov/chronicdisease/overview/index.htm

[51]
Centers for Disease Control and Prevention. Chronic Diseases: The Power to Prevent, The Call to Control: At A Glance 2009
http://www.cdc.gov/chronicdisease/resources/publications/AAG/chronic.htm

[52] DeVol, R , Bedroussian, A. The Milken Institute: An Unhealthy America. The Economic Burden of Chronic Disease. Oct 2007

[53] The Partnership to Fight Chronic Disease. Richard H. Carmona MD MPH FACS. 17th Surgeon General of the United States. 2008 Almanac of Chronic Disease.
http://www.fightchronicdisease.org/pdfs/PFCD_FINAL_PRINT.pdf

[54] Human Oral Microbiome Database. http://www.homd.org/

[55] Pollack, S., A. Mogtader, and M. Lange. 1984. Neisseria subflava endocarditis.Case report and review of the literature. Am. J. Med. 76:752-758.

[56] White, J C, and C FNiven. "Streptococcus s.b.e.: A Streptococcus Associated with Subacute Bacterial Endocarditis." Journal of bacteriology.1946. Volume 51 No. 6. p.717-22.

[57] Kamath U, Singer C, Isenberg HD, Clinical significance of Staphylococcus warneri bacteremia, J Clin Microbiol. 1992 Feb;30(2):261-4.

[58] Hospital-Acquired Pneumonia, The Merck Manuals Online Medical Library, 2008, http://www.merckmanuals.com/professional/sec05/ch052/ch052c.html

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