Dissertation

A STUDY TO ASSESS RESIDUAL MICROBIAL CONTAMINATION OF TOOTHBRUSH HEAD

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW,

UTTAR PRADESH In the partial fulfillment

of the requirement for the degree

Of

MASTER OF DENTAL SURGERY

In

PERIODONTOLOGY

By

DR CHETAN CHAUDHARY

Under the guidance of

DR MONA SHARMA

HEAD OF DEPARTMENT

Department of Periodontology

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,

LUCKNOW

(Faculty of Babu Banarasi Das University)

BATCH: 2019-2022

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BBD College of Dental Sciences
BBD Univeristy, Lucknow

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GUIDE

Dr Mona Sharma
Professor and Head of Department
Department of Periodontology
BBDCODS
BBDU, Lucknow (UP)

CO- GUIDE

4/4/2022

Dr Vandana A Pant
Professor
Department of periodontology
BBDCODS
BBDU, Lucknow (UP)

DECLARATION BY CANDIDATE

I hereby declare that the dissertation entitled "**A STUDY TO ASSESS RESIDUAL MICROBIAL CONTAMINATION OF TOOTHBRUSH HEAD**" is a bonafide and genuine research work carried out by me under the guidance of **Dr Mona Sharma** Head Of The Department, Department of Periodontology. Babu Banarasi Das College of Dental Sciences, Lucknow, Uttar Pradesh.

Date: 4/4/22

Place: Lucknow

Chetan

Dr Chetan Chaudhary

ENDORSEMENT BY HEAD OF DEPARTMENT

This is to certify that the dissertation entitled "**A STUDY TO ASSESS RESIDUAL MICROBIAL CONTAMINATION OF TOOTHBRUSH HEAD**" is a bonafide work done by **Dr Chetan Chaudhary** post graduate student, Department of Periodontology, under the guidance and supervision of **Dr Mona Sharma**, Professor, Depurtment of Periodontology. Babu Banarasi Das College of Dental Science, Lucknow, Uttar Pradesh.

DR MONA SHARMA

Professor and Head

Department of Periodontology

BBD College of Dental Sciences

, BBD University, Lucknow (U.P.)

ENDORSEMENT BY HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled " **A STUDY TO ASSESS RESIDUAL MICROBIAL CONTAMINATION OF TOOTHBRUSH HEAD**" is a bonafide work done by **Dr Chetan chaudhary** post graduate student, Department of Periodontology, under the guidance and supervision of **Dr. Mona Sharma**, Professor, Department of Periodontology. Babu Banarasi Das College of Dental Science, Lucknow, Uttar Pradesh.

Dr. Puneet Ahuja

Principal

Professor and Head

Department of Oral pathology

BBD College of Dental Sciences,

BBD University, Lucknow (U.P.)

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

ACKNOWLEDGEMENT

"No one who achieves success does so without acknowledging the help of others."

My mentor, **Dr Mona Sharma**, M.D.S. Professor and Head of the Department, Department of Periodontics, deserves the highest praise. Babu Banarasi Das College of Dental Sciences in Lucknow patiently supplied me with the vision, counsel, and motivation I needed to finish my dissertation. Her extensive expertise and abilities to attain greatness have proven to be quite beneficial throughout the process. I will be eternally grateful for her scholarly guidance.

It brings me great pleasure to express my profound gratitude to my esteemed Professor, **Dr Vandana A Pant**, a woman endowed with remarkable foresight and the ability to attain excellence via a wealth of knowledge. Thank you, ma'am, for always being an inspiration and believing in me even when I didn't believe in myself.

It brings me great pleasure to express my gratitude to my respected Principal, **Dr Puneet Ahuja**, for his permission and advice over the course of this project.

I am grateful to the Readers **Dr Sunil Verma**, **Dr Ashish Saini**, and **Dr Suraj Pandey**, as well as the Senior Lecturers **Dr Neelesh Singh**, **Dr Akansha Kashyap**, **Dr Himangi Dubey**, **Dr Meghna Nigam**, and **Dr Mohammad Aamir**, for their unwavering support and excellent ideas whenever I sought them.

Dr Subodh Kumar of the Birbal Sahni Institute of Palaeosciences Research Institute in Lucknow (U.P.) is to thank for allowing me to use the Scanning Electron Microscopy machine at their prestigious research institute.

I'd want to express my gratitude to my colleagues, **Dr Pallavi Goswami** and **DrDilip Kumar Maurya**.

My juniors for their support and assistance, we thank **Dr Ankit**, **Dr Shaifali**, **Dr Rahul Anand**, **Dr Snigdha**, **Dr Jigme Dr Sumati**, **Dr Akriti, Dr Arti, Dr bhibuti, Dr Deepika, Dr Dikshita, Dr Hiya** in the most critical moments From

the bottom of my heart, I am grateful to my mother, **Mrs Manju Chaudhary**, my father, **Mr Sant Prakash Chaudhary** for their blessings in allowing me to accomplish this work.

I am eternally grateful to my brother, **Dr Abhay Chaudhary** for his unwavering love, assistance, and support.

I owe my gratitude to my best friend **Dr Madina Ahmadi** for her unwavering love, support, and encouragement, as well as her best wishes.

BEST REGARDS

Dr Chetan chaudhary

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LIST OF ABBREVIATIONS

et al.	et alia (and others)
CFU	Colony Forming Unit
SEM	Scanning Electron Microscopy
Pd	Palladium
Pt	Platinum
NACL	Sodium Chloride
psi	Pound-force per square inch
pa	Pascal
ml	milliliter
nm	Nanometer
Pvt. Ltd	Private and Limited

LIST OF ANNEXURES

ABSTRACT

Brushes have been an important part of oral hygiene for ages. The retention and survival of microorganisms on toothbrushes after brushing is a potential source of mouth re-contamination. As a result, they play an important role in disease transmission and raise the risk of infection since they might serve as a microorganism reservoir. Dental caries, gingivitis, stomatitis, and infective endocarditis have all been linked to these microorganisms, compromising both oral and general health. As the toothbrush is used more frequently, the bristle thickness varies, affecting plaque control. The goal of this study was to determine the microbial load between the bristle tufts on the toothbrush head and to measure bristle thickness. The study focused on toothbrushes, and 50 toothbrushes with the same specifications were distributed at random to undergraduate students at the same university, with two groups: Group A (3 months) and Group B (3 months) (4 months). The undergraduate students were especially included since the toothbrushes could be recovered from them. The Toothbrush head and Toothbrush Bristles were analysed for Colony Forming Units and Scanning Electron Microscopy after 3 and 4 months. The study found that Group B had a higher number of Colony Forming Units than Group A, and that there was a considerable thickness difference between Group B and the unused toothbrush bristles and Group A.

INTRODUCTION

Oral health is an important aspect of general health, It demonstrates one's overall well-being,hence, maintaining good oral hygiene becomes imperative.Dental plaque is the predominant causal factor in both gingivitis and periodontitis. Good oral hygiene by effectively brushing the teeth plays a salient role in maintaining oral health, which prevents periodontal diseases and dental caries. However,a toothbrush and other oral hygiene aids every day is the most reliable way to achieve oral health benefits for all. Contaminated toothbrushes have been linked to a variety of oral and systemic disorders, including septicemia, as well as gastrointestinal, cardiovascular, pulmonary, and renal issues. This is especially crucial for youngsters, the elderly, and high-risk patients, such as those who are immunocompromised or undergoing organ transplantation or chemotherapy. A toothbrush and other oral hygiene aid every day is the most reliable way to achieve oral health benefits for all.

As a toothbrush is used longer, the microorganism scolonize in between the bristles despite proper maintenance of the brush. The contamination occurs from the environment, hands, aerosols and storage containers. In addition, these microorganisms from the bristles are capable of transmiting disease to an individual[1,2] and can recontaminate the oral cavity and at times, resulting in infections such as gingivitis and stomatitis[3]. When used for longer duration, the surface area and the flare of the bristles increase, thereby decreasing the efficiency of the toothbrush. Bristle fields are often used as indicators of bristle bending; the bigger they are after use the farther the bristles have bent. The assessment of the bristle field area increase is commonly used to determine the degree of wear of a brush[4,5]. When inspecting used toothbrushes, apparent symptoms of wear are frequently associated with poor cleaning efficiency in terms of plaque removal[6,7] or gingival injuries caused by split ends in the bristles[8,9].

SEM (Scanning Electron Microscopy) has shown to be a valuable research tool. It is one of the most widely used techniques for examining the microstructure and morphology of materials and structures. Images at high magnifications of 50 to 10000 magnification and above can be visualized with SEM. When a focused stream of electrons strikes the specimen's surface topography, it creates a variety of waves (the waves produced depends upon the type of the specimen). The detector collects the feedback. As a result, the exact surface topography of the specimen is revealed.

Higher atomic number regions appear darker and lower atomic number regions appear lighter.

Hence, the purpose of the present study was to assess the duration a toothbrush can be safely used for. The basis of this duration was scientifically based upon the fact of microbial colonization occurring in the bristles of the toothbrush with use that was assessed by microbiological culturing. There by keeping in mind both these factors the duration a toothbrush should be used for, was analyzed. Also, changes in the dimensions of the bristle was found, that was corroborated by SEM study.

AIMS AND OBJECTIVES

AIM

1. To establish the microbial load in between the bristle tufts on the head of the toothbrush.

OBJECTIVES

1. To establish Colony Forming Units (CFU) on toothbrush head after 3 months of toothbrush use.
2. To establish Colony Forming Units (CFU) on toothbrush head after 4 months of toothbrush use.
3. To assess bristle thickness at baseline (Unused), after 3 months and after 4 months.
4. To evaluate a co relation between bristle thickness and Colony Forming Units.

REVIEW OF LITERATURE:

1. **S. S. TAJI et al (1998)[10]:** did a clinical study in which thesubjects were each supplied with toothbrush(same type and brand), with fluoridated toothpaste. After a three-week period, during which subjects were asked to follow their usual oral hygiene practices, the toothbrushes were collected and assayed for microbial contamination using a range of selective growth media. The total microbial load per toothbrush was found to be 10' to 106 colony forming units. Staphylococci were found on all toothbrushes and streptococci on all but one. These two genera were also quantitatively dominant. Candida, corynebacteria, pseudomonads and coliforms were identified in 70, 60, 50 and 30 per cent of toothbrushes, respectively. However, mutans streptococci, lactobacilli and black-pigmented Gram-negative anaerobic rods were not detected on any of the toothbrushes. For each individual, information on variables such as toothbrush rinsing practices and post-brushing storage methods and environment was collected. No obvious relationship between such variables a microbial load was apparent but it was suggested that more extensive studies are needed, taking into account additional parameters such as age and degree of toothbrush.
2. **W. WETZEL et al (2005)[11]: S**tated that the retention and growth of cariogenic microorganisms on toothbrushes pose a threat of recontamination. The authors studied three species of oral microorganisms found at different places on toothbrush filaments. The authors tested on 30 patients with different toothbrushes made by a single manufacturer. The toothbrushes were divided into three groups by type of construction: staple set tufting (toothbrush A); in-mold tufting (toothbrush B); individual in-mold placement of filament (toothbrush C). Subjects used the toothbrushes once under standardized conditions; the authors subsequently examined the brushes for the presence of *Streptococcus mutans,* lactobacilli and *Candida* species. The inspection was carried out at three-time intervals after use.
3. **J. R. SUKHABOGI et al (2015)[12]:** They did a study **t**o qualitatively and quantitatively assess the microbial contamination of tooth brushes preserved in different sanitary settings before and after disinfection with 0.2% chlorhexidine.The study was carried out in two phases among thirty participants visiting a dental hospital. These participants were assigned to one of the three

groups based on the practice of preserving the tooth brush. Group 1: Participants who preserved their brush outside the bathroom. Group 2: Participants who preserved their brush within the bathroom without attached toilets. Group 3: Participants who preserved their brush within the bathroom with attached toilets. Participants were given oral hygiene kits containing a brush and paste in the first phase. The brush samples were collected on day thirty for qualitative and quantitative estimation of microbial contamination. In the second phase, participants were requested to rinse their brushes in 0.2% chlorhexidine after brushing and before placing it back. The mean CFU of different bacteria was compared using independent sample t-test and paired sample t-test.

4. **C. MCCARTH et al (1965)[13]:** In this clinical study they evaluated the oral samples obtained from a group of fifty-one newborn and forty-four-month-old infants and repeat specimens collected from the latter group at the ages of 8 and 12 months yielded a total of 153 oral specimens. The incidence of thirteen bacterial genera was determined. Only species of Streptococcus, Staphylococcus, Veilionella and Neisseria were constantly present by 12 months of age; Actinomyces, Lactobacillus, Nocardia and Fusobacterium species were cultured in more than half the subjects at this age, while Bacteroides. Leptotrichia, Candida, Corynebacterium species and the coliform types were isolated from less than half of these infants. Quantitatively, the average total bacterial counts were of the order 104-5 per milligram of sample. Streptococci were dominant but the percentage diminished from 98 per cent to 70 per cent of the total by the end of the first year of life. The accretion of filamentous and branching forms was found to occur with advancing age but did not appear to be solely dependent upon tooth eruption since these forms could be isolated from predentate infants.

5. **R. BOYLEN et al (2008)[14]:** In this study they evaluatedthis two armed, self-controlled, investigator blinded, clinical study tested the efficacy of an ultraviolet (UV) light toothbrush holder (Violight) to decrease toothbrush bacterial contamination. 25 subjects were randomly assigned to control or experimental groups and received two toothbrushes for home use on either even or odd days. The control group rinsed both toothbrushes after use in cold tap water with no mechanical manipulation. The experimental group rinsed one toothbrush in cold running water while storing the other toothbrush in the Violight toothbrush holder

after use. The toothbrushes were returned after 2 weeks use in sealed plastic bags and were analyzed for the number of colonies forming units (CFU) of S. mutans, S. salivarius, lactobacilli, E. coli, and other coliforms, and total bacterial counts by culture. An additional analysis of the total bacterial profile was performed using denaturing gradient gel electrophoresis (DGGE).

6. **S. D. Caudry et al. (1995)[15]:**Twenty toothbrushes used by healthy subjects were screened for the presence of microorganisms. Microbes were dislodged from the brushes by vortexing, and an average of 4 x 10(3) CFU/mL were recovered from the suspending fluid. Bristles removed from the vortexed brushes still yielded confluent bacterial growth on brain-heart infusion agar medium. Virkon (one per cent), Listerine, Cepacol, Scope, and Plax were tested for their bactericidal effects on microorganisms sedimented from the suspending fluid, on toothbrush bristles and proxabrushes, and on various test species including Candida albicans, Mycobacterium smegmatis, M. bovis, and Streptococcus mitis. Virkon and Listerine killed all the test species and virtually all the microorganisms on the toothbrush bristles and proxabrushes. Six volunteers tested the efficacy of a Listerine soaking regime to prevent the bacterial contamination of toothbrushes. Soaking the toothbrush head (bristles) in Listerine for 20 minutes after brushing was sufficient to eliminate bacterial contamination.

7. **R. T. Glass and M. M. Lare(1986)[16]:** Toothbrushes are commonly used to remove dental plaque, and the presence of bacteria on toothbrushes has been previously reported. Toothbrushes are contaminated by many bacteria after brushing, and contaminated toothbrushes can cause oral and systemic diseases. Toothbrush contamination was studied previously, but the study was limited because it only identified specific bacteria using a general bacterial culture method. To overcome this limitation, we used Illumina sequencing to identify microorganisms present on toothbrushes. Toothbrush samples were divided into two groups according to the storage location: a toothbrush stored in the once or a toothbrush in the bathroom. Samples were sequenced using Illumina sequencing. Enterococcus (30.76%), Pseudomonas (21.85%), Streptococcus (14.94%), and Lactobacillus (5.15%) were the predominant bacteria found on the toothbrushes stored in the once. Streptococcus (19.73%), Pseudomonas (16.08%), Enterococcus (8.16%), and Neisseria (7.04%) were the predominant species on the toothbrushes

stored in the bathroom. In addition, 36.29% of the bacteria on the toothbrushes stored in the once and 33.77% of the bacteria on the toothbrushes stored in the bathroom were identified as potentially pathogenic bacteria. Both groups included microorganisms such as Streptococcus, Actinomyces, Porphyromonas, and Fusobacterium that are related to oral disease. This study confirmed the high contamination rate of used toothbrushes and demonstrated that repeated use of toothbrushes could lead to contamination by pathogenic bacteria.

8. **N. Grewal and K. Swaranjit et al (1996)[17]:**Used toothbrushes for varying time periods are collected from students. Then their bristles are plucked with the sterilized forceps for the prevention of bacterial contamination and agitated in the saline and inoculated in the brain heart infusion broth agar with the help of sterilized bacterial loops and incubated for 24 hours at 37oC. Then the colonies are counted and record as CFU/ml.

9. **L. Bunetel et al (2000)[18]:** The retention and survival of microorganisms on toothbrushes pose a threat of recontamination for certain patients at risk. In order to measure the influence of brush design and optimize the choice of toothbrush model for complementary studies, the in vitro retention of three microbial species (Porphyromonasgingivalis ATCC 33277, Streptococcus mutans ATCC 25175 and Candida albicans ATCC 26555) was evaluated for three types of toothbrush. Two series of standardized experiments were carried out for each brush and microorganism. The first series tested the retention of the microorganisms on the head portion of the brush, while the second measured retention on the head of the brush and the part of the handle inserted in the mouth during brushing. For each series, the microorganisms were counted at T0 and T24 (after storage of the brushes at room temperature for 24 h). Depending on the microorganism studied, from 0.2% to 2% of the initial inoculum was retained on the brush. The number detected increased with the size of the exposed area. After 24 h, P. gingivalis and S. mutans were found on only one type of brush. C. albicans survived on all three. These results confirm that microorganisms can quickly colonize toothbrushes.

10. **R. T. Glass et al (1994)[19]:** Sixty-six sterile toothbrushes were exposed to one of the following microorganisms: Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillis subtilis, Serratia marcescens and Baker's yeast. The Pollenex DS60 Daily Dental Sanitizer was found to be effective in substantially

reducing the number of retained bacteria and yeasts as compared to contaminated toothbrushes not treated with such a device. Different toothbrush types had different response rates. Seventy-two sterile toothbrushes were exposed to Herpes Simplex Virus, Type I and seventy-two sterile toothbrushes were exposed to Parainfluenza Virus, Type III. The Pollenex DS60 Daily Dental Sanitizer consistently killed both viruses on all of the toothbrushes treated. Both viruses were consistently retained on non-treated toothbrushes for at least 24 hours.

11. **A. Mehta et al (2007)[20]:** The purpose of this study was to determine the extent of bacterial contamination of toothbrushes after use and the efficacy of chlorhexidine and Listerine in decontaminating toothbrushes. The effectiveness of covering a toothbrush head with a plastic cap in preventing contamination was also evaluated. It was found that 70% of the used toothbrushes were heavily contaminated with different pathogenic microorganisms. Use of a cap leads to growth of opportunistic microorganisms like Pseudomonas aeruginosa, which may cause infection in the oral cavity. Overnight immersion of a toothbrush in chlorhexidine gluconate (0.2%) was found to be highly effective in preventing such microbial contamination.

12. **J. Verran et al (1996)[21]:** Used brushes (28 in toto) were assessed for microbial contamination. The micro-organisms removed from the toothbrush heads were plated onto a range of selective media. The total number of micro-organisms isolated per brush varied from 0 to 10(8) CFU. Staphylococci, coliforms, pseudomonads and yeasts were isolated from 64, 57, 28 and 39% of brushes, respectively. Identification tests on representative colonies indicated that media for streptococci, staphylococci, yeasts and pseudomonads were selecting for appropriate growth with > 90% efficiency. Of those tested on MacConkey agar eight from eleven colonies were oxidase negative, Gram-negative rods; the remainder were oxidase positive. No black pigmented obligate anaerobes were isolated. None of the seventeen colony types on Helicobacter selective agar proved to belong to that genus. Scanning electron microscopy of bristles revealed toothpaste debris, but micro-organisms were not evident.

13. **M. Efstratiou et al (2007)[22]:**They evaluated the effectiveness of alternative methods for toothbrush disinfection. Methods. Two-hundred eighty toothbrushes were included in the study. The toothbrushes were divided into 7 groups and were

contaminated by standardized suspensions of Lactobacillus rhamnosus (L. rhamnosus), Streptococcus mutans (S. mutans), Staphylococcus aureus (S. aureus), and Escherichia coli (E. coli). The following disinfectants were tested: 1% sodium hypochlorite (NaOCl), 100% and 50% white vinegar, microwave (MW) oven, ultraviolet (UV) sanitizer, and mouth rinse-containing propolis (MCP). Data were analyzed with Kruskal Wallis and Dunn's tests.Statistically significant differences were found between different methods and control group for all tested bacteria. There were statistically significant differences between all test groups for all microorganisms. MW was the most effective for L. rhamnosus and 100% white vinegar was the most effective method for S. mutans and S. aureus. NaOCl was the most effective for E. coli. Conclusion. This study showed that 100% white vinegar was considered to be effective for tested microorganisms. Similarly, 1% NaOCl is cost-effective, easily accessible, and comparatively effective for toothbrush disinfection. Because these agents are nontoxic, cost-effective and easily accessible, they may be appropriate for household use.

14. **S. Sato et al (2005)[23]:** Three different solutions were sprayed on toothbrush bristles among 30 adults after they had brushed: (1) basic formulation (base) plus chlorhexidine; (2) base only, and (3) sterile tap water (control). Each solution was tested for 1 week. After that, the toothbrushes were collected and sonicated in Letheen Broth, diluted in 10-fold series, and plated on selective and non-selective media for detection of anaerobes, aerobes, streptococci, and gram-negative bacilli. After incubation, the colonies of those microorganisms were counted. Presence of mutans streptococci on the bristles was also confirmed. RESULTS: Spray 1 produced a significant reduction in the microbial contamination of toothbrushes for all the microorganisms, spray 2 provided some reduction of contaminants, and spray 3 demonstrated the least anti-microbial effect.

15. **D. P.Warren et al (2001)[24]:** Twenty patients who had Type III or Type IV periodontitis participated in this study. One side of each of their mouths served as a control (no toothpaste). The teeth on the other side were brushed with a regular toothpaste or a triclosan-containing toothpaste. After the toothbrushes were allowed to dry in air for four hours, the authors placed the toothbrush heads in solution, dislodged the microbes from the brushes by vortexing and plated them in culture dishes. The authors anaerobically incubated the culture dishes and

determined the presence or absence of Prevotella species or Ps; Porphyromonasgingivalis, or Pg; and Actinobacillusactinomycetemcomitans, or Aa. Results. The authors detected Aa and Pg on the control toothbrushes more frequently than they did Ps. This variation in isolation frequency was statistically significant by $\chi 2$ analysis ($P < .001$). The authors compared the isolation frequency of the three test organisms between the control and regular toothpaste groups, between the control and triclosan-containing–toothpaste groups, and between the triclosan-containing–toothpaste and regular-toothpaste groups. They found no significant intergroup differences in the isolation frequencies after using $\chi 2$ analysis.

16. **M. C. Goldschmidt et al (2004)[25]:** Twenty patients had one side of their mouths brushed with a toothbrush containing the antimicrobial agent (experimental side), and the other side with a toothbrush containing no agent (control). Toothbrushes were air-dried (25 degrees C) for four or 24 hours. Toothbrush heads were vortexed and cultured for Prevotella species (Ps), Porphyromonasgingivalis (Pg), Actinobacillusactinomycetemcomitans (Aa), and non-specific colony-forming units (NS). The plates were incubated and counted. Means and standard deviations were calculated, and data were analyzed using a series of t-tests (paired and unpaired) and Wilcoxon matched-pairs signed-rank test. Results: No significant inter- or intra-group differences in mean counts were found; however, when four-hour and 24-hour data for Aa, Pg, or NS were combined, experimental counts were lower than controls in 39/50 (78%) of the matched pairs (Wilcoxon signed-rank test $p = 0.01$).

17. **M. loitongbam et al (2021)[26]:**In total, 160 samples comprising of 80 enamel and cementum each were equally and randomly divided into four groups: Group 1 multi-directional powered toothbrushing; Group 2 oscillating/rotating/pulsating powered tooth brushing; Group 3 sonic powered tooth brushing; and Group 4 manual tooth brushing. They were further sub-divided equally into Control and Test. The Test samples were brushed for 2 min every day for a period of 1 month. The prepared samples were evaluated for surface roughness using scanning electron microscope at 1000 × magnification and atomic force microscope at the nanoscale. Result: A statistically significant difference was seen in the enamel and cementum roughness between multi-directional tooth brush group and sonic (P =

0.00); multi-directional tooth brush group and manual tooth brush group (*P* = 0.00); oscillating tooth brush group and sonic group (*P* = 0.00); oscillating tooth brush group and manual tooth brush group (*P* = 0.00); and sonic group and manual tooth brush group (*P* = 0.00).

18. **Apiou J et al. (1994)[27]:**Comparedthe efficacy of a new toothbrush filament layout concept (Topix, Peridental, France) to that of a standard vertical-tuft toothbrush. Bacterial and exogenous deposit elimination were used as parameters of efficacy. 30 dental surgery students took part in the study. Plaque index scores were calculated according to a pre-defmed protocol. Imprints of the 6 anterior teeth were taken before and after brushing with the 2 types of brushes, without toothpaste or rinsing. Imprints were examined by scanning electron microseopy (SEM) 12 h after brushing, imprint examination revealed bacterial flora polymorphism and the amount of dental plaque accumulated at the cervieai third zone of teeth. Automated quantification in this zone of exogenous bodies showed that after brushing with vertical-tuft and cross-tuft brushes, there remained 1 *76 mrrr*and 0.83 mm- of dental plaque, squamae, and blood residues, respectively The plaque index values correlated to scanning electron microscopic observations. There was no significant difference in terms of efficacy between the cross-tuft and vertical-tuft toothbrushes.

19. **Checchi Let al (2001)[28]**: Did this study to evaluate the % of rounded filaments considered to be of acceptable quality in different toothbrush brands and to determine whether there is a standardization of quality, as manufacturers claim. Brushes tested included 2 samples of medium-hard nylon or tynex toothbrushes from 31 various types found on the retail market in Italy. Tufts from the same position on the toothbrush head were removed and examined under a stereomicroscope, utilizing methods which did not alter the physical properties of the filaments. In 4 of the 31 toothbrush brands tested, more than 50% of the filaments appeared rounded, in 19 of them, between 11.9% and 40.5% and in 8 brands between 0% and 7%. Differences were found in the number and disposition of filaments among different brands and even within the same toothbrush brand. The results appear to indicate that a large % of toothbrushes on the retail market do not meet acceptable quality criteria.

20. **Karen B. Williams et al (2001)[29]**: The purpose of this study was to evaluate the effects of both a sonic and a mechanical toothbrush versus the effects of no treatment on depth of subgingival penetration of epithelial and tooth associated bacteria. Eight adult subjects exhibiting advanced chronic periodontitis with at least 3 single-rooted teeth that were in separate sextants with facial pockets 4 mm and ≤ 8 mm and that required extraction constituted the experimental sample. Teeth were either subjected to 15 seconds of brushing with a mechanical toothbrush or a sonic toothbrush or left untreated. The test tooth and the associated soft tissue wall of the periodontal pocket were removed as a single unit. Samples were processed and coded for blind examination by scanning electron microscopy. Distributional and morpho logic characteristics of dominant bacteria with specific emphasis on spirochetes were evaluated for both epithelial- and tooth-associated plaque. Results: No differences were found in morphotypes or distributional and aggregational characteristics of epithelial-associated microbes in the 1- to 3-mm subgingival zone between the mechanical and sonic toothbrush-treated groups and the control group. Both toothbrush groups featured disruption of microbes that extended up to 1 mm subgingivally. Root surfaces on the sonic-treated samples appeared plaque-free at low magnification; however, at 4,700x, a thin layer of mixed morphotypes and intact spirochetes was found supragingivally and slightly subgingivally. In comparison, mechanical brush samples featured incompletely removed plaque, both supragingivally and subgingivally, with intact spirochetes present on subgingival root surfaces.

21. **Paulo Nelson-Filho et al (1999)[30]**: In this study they evaluated the level of contamination of toothbrushes by mutans streptococci using microbiological identification, to access the bacterial contamination using scanning electron microscopy (SEM) and to evaluate the efficacy of two toothbrush disinfectants. Nineteen children used their toothbrushes once a day, for five consecutive days. The toothbrushes were then immersed into disinfectant solutions for 20 h: Group I--0.12% chlorhexidine gluconate; Group II--1% sodium hypochlorite; Group III--sterile tap water. They were then placed into test tubes containing CaSa B, for 3 to 4 days at 37 degrees C. The number of MS CFU was counted and the toothbrushes were submitted to SEM analysis. There was no bacterial growth in Groups I and II; Group III showed MS growth (range, 21 to 120 CFU). Scanning electron

microscopy showed biofilm formation on toothbrush bristles. Immersion in 0.12% chlorhexidine gluconate and 1% sodium hypochlorite are efficient methods for toothbrush disinfection.

22. **H-S Lee et al (2015)[31]:** Theyevaluated the proportions of end-rounded bristles via observations of the end patterns of various children's toothbrushes with scanning electron microscopy (SEM) and stereomicroscopy.Ten different brands of children's toothbrushes were chosen, and tufts from each toothbrush were used. The prepared bristle specimens were observed on SEM and stereomicroscopic images and classified as acceptable (A1–A3) and non-acceptable (N1–N5) according to the modified classification. Then, the proportions of end-rounded bristles were calculated.Analyses of the 10 toothbrushes revealed that the proportions of acceptable end-rounded bristles ranged from 1.4% to 20.2% on SEM and from 0.0% to 18.0% on stereomicroscopic examinations. Additionally, some toothbrushes had labels that indicated bristle end-rounding, but the proportions of end-rounded bristles were low.

23. **Ojha S.K. et al (2015)[32]**: They did this study to evaluate the abrasivity of the toothbrush versus the velcro fasteners. Forty extracted clinically healthy premolars were grouped in two groups. Group A comprising of teeth that were subjected to toothbrush bristles and group B to velcro fasteners (hook and loop). The mounted teeth of both the groups were subjected to abrasion test, and the tooth surfaces were observed for the possible abrasions from the oscillating strokes (toothbrush) and frictional contacts (hook and loop velcro) and examined under the scanning electron microscope. Comparative assessment of both velcro (hook and loop) and toothbrush bristles did not reveal any evidence of abrasion on the tooth specimens.

24. **Nüss K.V. et al (2010)[33]:** They examined the differences in wear in manual toothbrushes from different price categories. 140 volunteers (14 groups of 10) brushed twice daily for 2–3 minutes over a period of three or six months using the modified Bass technique and seven different toothbrushes (TB) from three price categories. A: 2 TB for under 1 Euro; B: 2 TB priced between 1 and 2 Euro; C: 3 TB priced at over 2 Euro. After a period of three or six months the increase in the bristle surface field was determined and the brush heads were rated macroscopically, by light microscopy and scanning electron microscopy (SEM) (grades 1–4: new, small, clear or very clear signs of use). The statistical analysis

was performed with the Mann-Whitney U-test and Error Rates method (p 0.05). All bristle fields showed an increase in surface area over the period of use. When examined macroscopically and under light microscopy, very little difference was found between three and six months of use, or between brushes from the same price category. The clearest distinction was found between categories B and C, whereby C was rated worse. In SEM it was difficult to separate the findings according to price categories. Here, the scores most often awarded were 3 and 4. The results of the three test methods differed markedly from one another. Thus, no conclusions on the state of the bristles can be drawn from a marked increase in bristle field surface area. The category B TB tended to perform best.

MATERIALS AND METHOD

This study was conducted in the Department of Periodontology of BBDCODS, BBDU, Lucknow and Birbal Sahani institute of Palaeosciences Research Institute, Lucknow.

Patients were selected from the OPD of the department based upon the following inclusion and exclusion criteria.

Inclusion criteria: -

- Participants aged between 18-28 years.
- They should be free from any oral and/or systemic disease.
- Should not have undergone Scaling and root planing in the last 3 months.
- They should not have used any mouthwash in the last 3 months.

Exclusion Criteria: -

- Participants with any oral and/or systemic disease

MATERIALS AND EQUIPMENT USED IN THE STUDY: -

Armamentarium For Microbial Analysis: -

- Toothbrushes medium bristles
- Blood agar culture media
- plastic sterile containers
- inoculation loops
- micropipette
- test tubes

Armamentarium For SEM Study: -

- Toothbrush medium bristles
- Scanning Electron Microscope

Methodology:

The study was carried out in the of Dept. of Periodontology, BBDCODS, Lucknow. Subjects selected were undergraduate students of the same Institute. They were specifically selected for the study purpose since retrieval of the toothbrushes would be possible from them. Medical history and Clinical examinationswere carried outthose fulfilling the inclusion criteria were selected. 50 toothbrushes of the same specifications were then randomly distributed to the subjects given to undergraduate

students of the same institution. The undergraduate students have been specifically included since the retrieval of the toothbrushes from them would be possible.

The subjects were randomly divided into 2 groups: -

Group A-25 participants, were asked to use the toothbrush for 3 months.

Group B-25 participants, were asked to use the toothbrush for 4 months.

The participants were taught and asked to follow only modified bass brushing technique. They were instructed to refrain from using any mouthwash for the duration of the study and were told to rinse with water only.

At the end of 3 months, the used tooth brushes of participants of Group A were collected in plastic sterile pouches to keep the toothbrushes sterile. Similarly, it was done for Group B participants at the end of 4 months. Upon collection of the toothbrushes, the toothbrush heads were separated from their handles using a straight hand piece and a disc bur, collected, and stored in saline medium under sterile conditions

Microbial Assessment Procedure: -

To begin 100 ml of 0.85% NACL solution (Saline) was prepared. Serial dilution of the prepared solution was done by transferring 10 ml in first test tube and 9 ml in rest of the nine test tubes, this serial dilution method is based upon the principle that when a material containing microorganisms is cultured, each viable organism will develop into a Colony. Hence, the number of colonies appearing on the plates represent the number of viable organisms in the sample. This technique which is also used for the isolation of bacteria, and to get ever the least number of bacterial colonies. Then the saline containing test tubes were autoclaved at 121°C/15 psi for 15 minutes. After that, using the micropipettes, the samples were transferred from the test tube to the culture plates. Blood agar was used as the culture media. The plates were then cultured at 30°C in an incubator for 24 to 48 hours. The culture plates were then seen under an automated colony counter. Colony Forming Units were estimated by using the formula: cfu/ml = (no. of colonies x dilution factor) / volume of culture plate.

In addition, Scanning Electron Microscope (SEM) study was also done to assess the thickness & morphology of bristles at:

1. Before use (Unused)
2. After 3 months
3. After 4 months

Before placing it under the SEM, bristle tufts were carefully separated from the toothbrush head by BP blade and were placed on to the stub.

Scanning Electron Microscope Procedure:

To resist a high vacuum (103 Pa), the samples (severed bristles) were thoroughly dried and were placed on a double-sided sticky tape on a metallic mounting surface that measured roughly 12.0 mm x 12.0 mm in length and width. The purpose of putting tufts on sticky tape was to prevent them from dispersing and thereby to proper visualize on the SEM. It was then placed inside a sputter coater machine, where all of the samples were automatically coated with a thin layer of conductive metal palladium and platinum alloy coating (Pt/Pd) ranging from 20 nm to 30 nm. The purpose of coating the specimen is to improve its conductivity in the Scanning Electron Microscope while also preventing the build-up of high voltage charges on it. The samples were then taken from the sputter coater and scanned using a Field Emission Electron Scanning Microscope (FESEM) (JEOL JSM 76610, JEOL India Pvt. Ltd.). Each sample was mounted on a new stump holder that was 12.0 mm x 12.0 mm in length and width and screwed tightly onto the stump holder. After that, the sample were placed in a vacuum chamber (as electrons do not travel very far in the air). The electron beam runs via a series of coil-shaped electromagnets in place of the FESE microscope's lenses. The image thus obtained is either a photograph (known as an electron micrograph) or an image on a television screen. The whole control and test surface of each specimen were scanned initially to provide a broad picture of the surface topography of each specimen.

SEM was used to investigate the toothbrush bristle thickness at baseline (unused), after 3 months and after 4 months.

Figure 1:Toothbrush

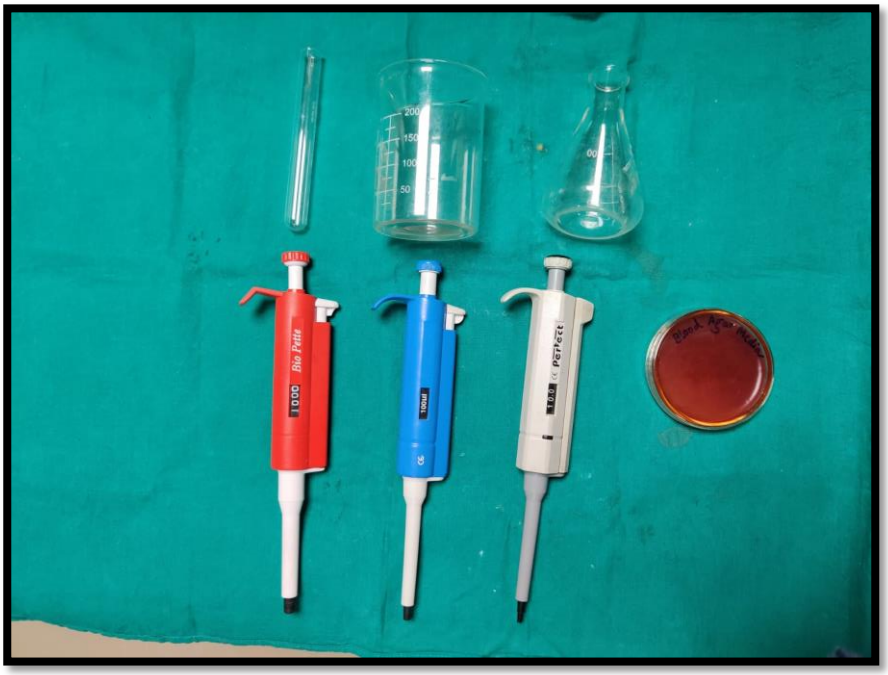


Figure 2: Armamentarium for sample preparation.

Figure 3: Incubator

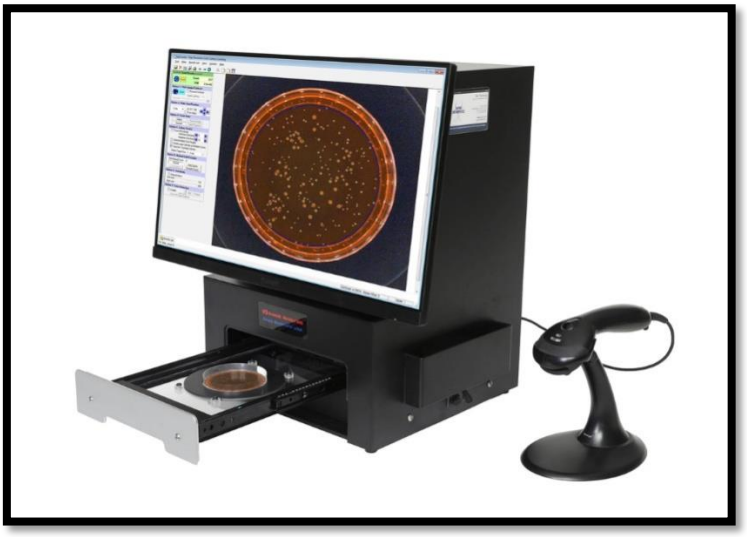


Figure4: Automated Colony Counter

Figure5: Toothbrush Bristles mounted for Sputter Coating with Palladium-Platinum Alloy

Figure6: Sputter Coater Machine

Figure7: Toothbrush Bristles Following Palladium-Platinum Coating

Figure8: Field Scanning Electron Microscopy.

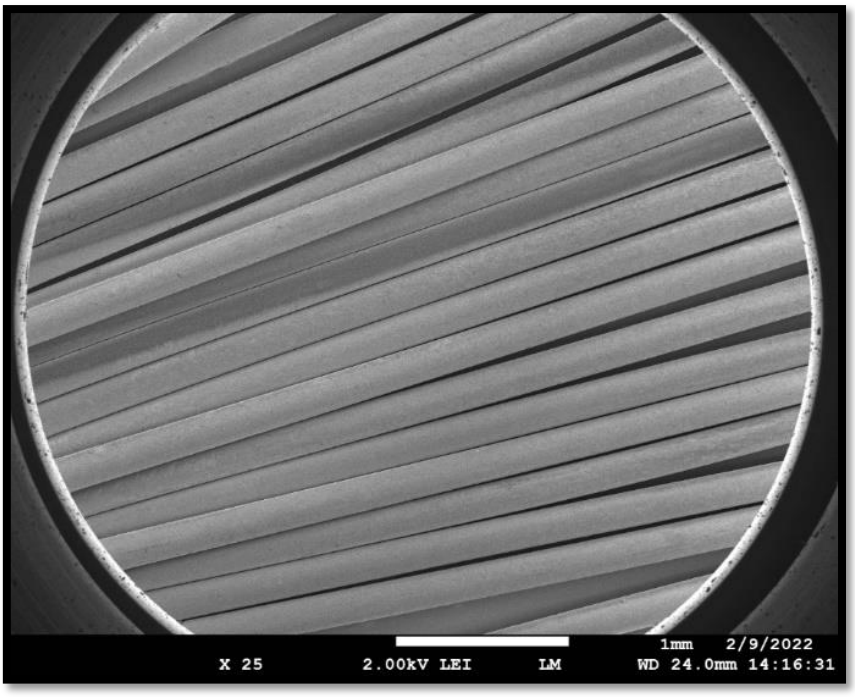


Figure 9: Unused Toothbrush Bristles at 25x Magnification

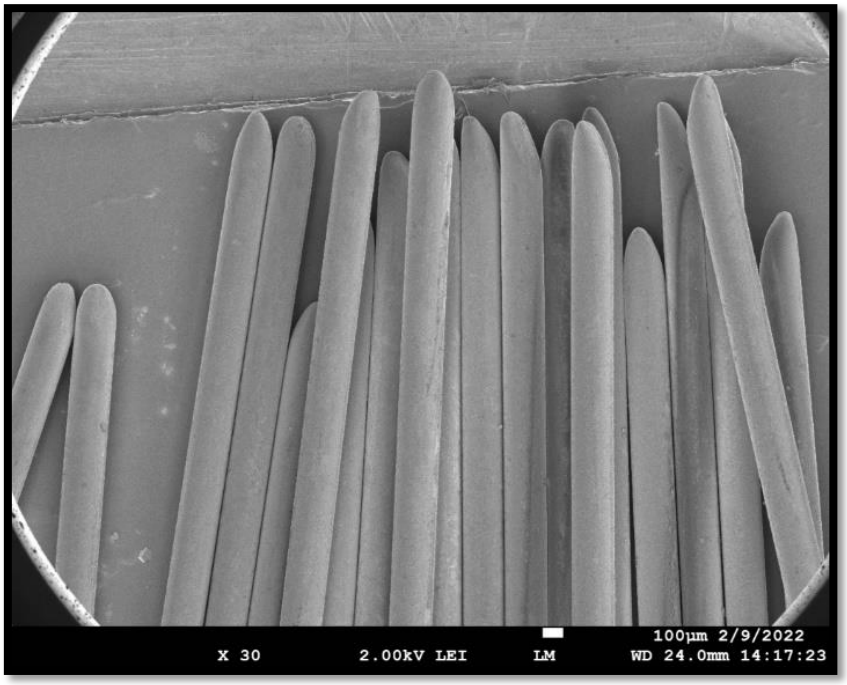


Figure 10: Unused Toothbrush Bristles at 30x Magnification

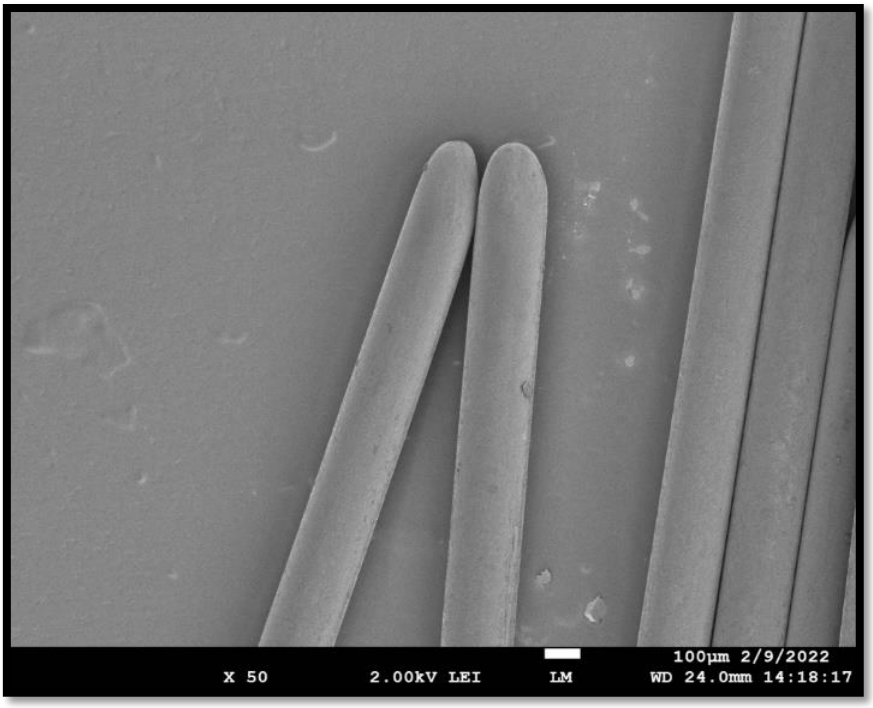


Figure 11: Unused Toothbrush Bristles at 50x Magnification

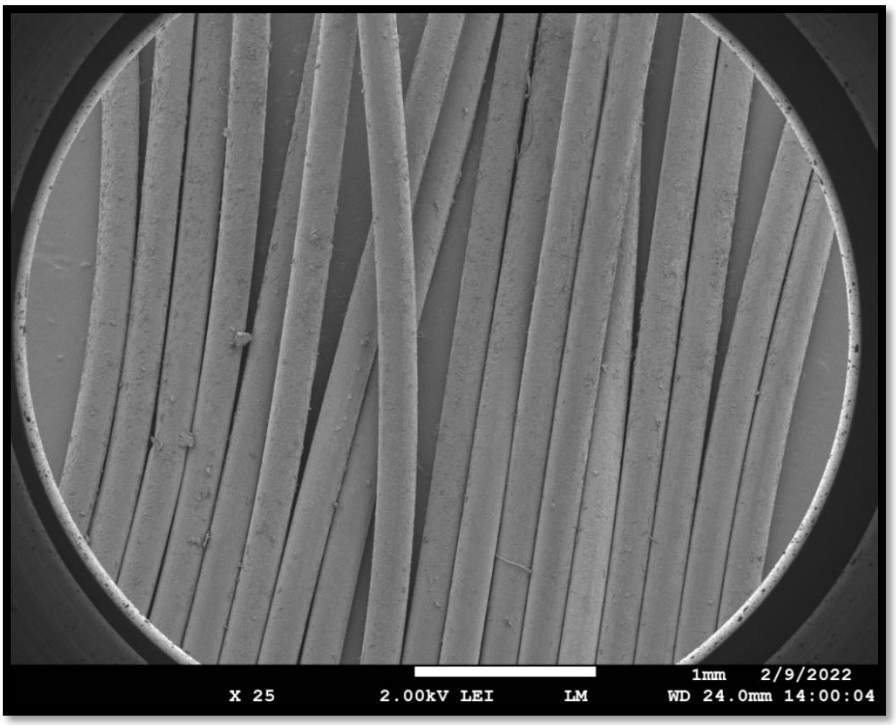


Figure 12: Group A- Toothbrush Bristles at 25x Magnification.

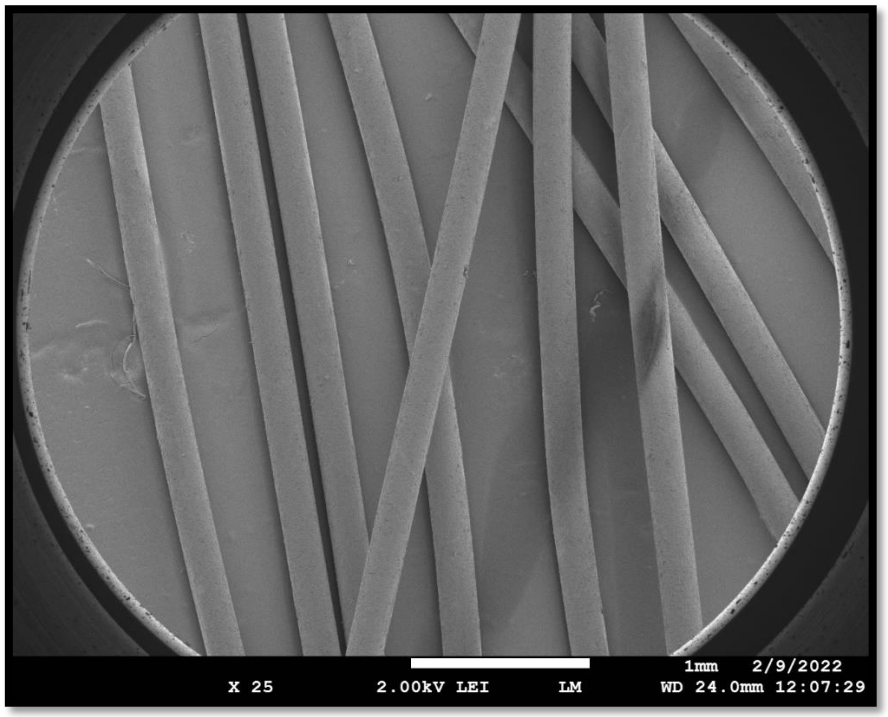


Figure 13: Group B Toothbrush Bristles at 25x Magnification.

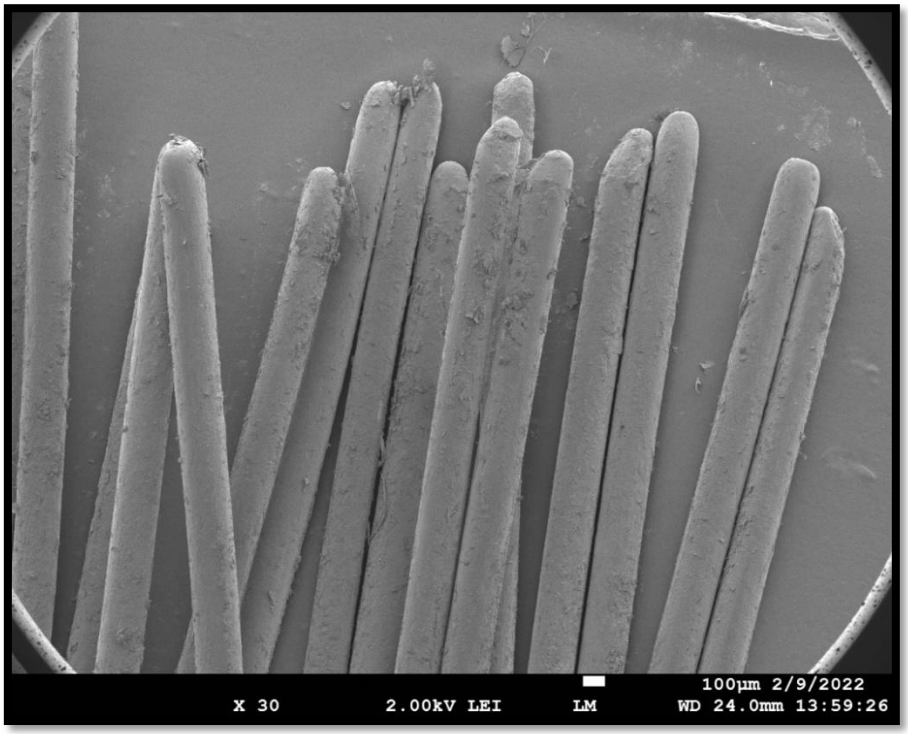


Figure14: Group A- Toothbrush Bristles at 30x Magnification.

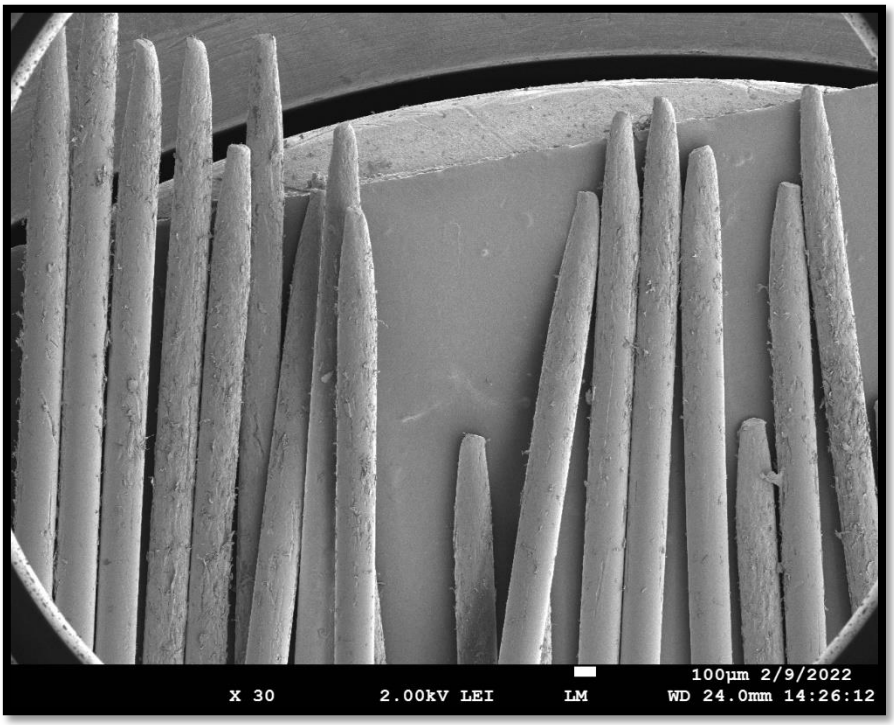


Figure 15: Group B- Toothbrush Bristles at 30x Magnification.

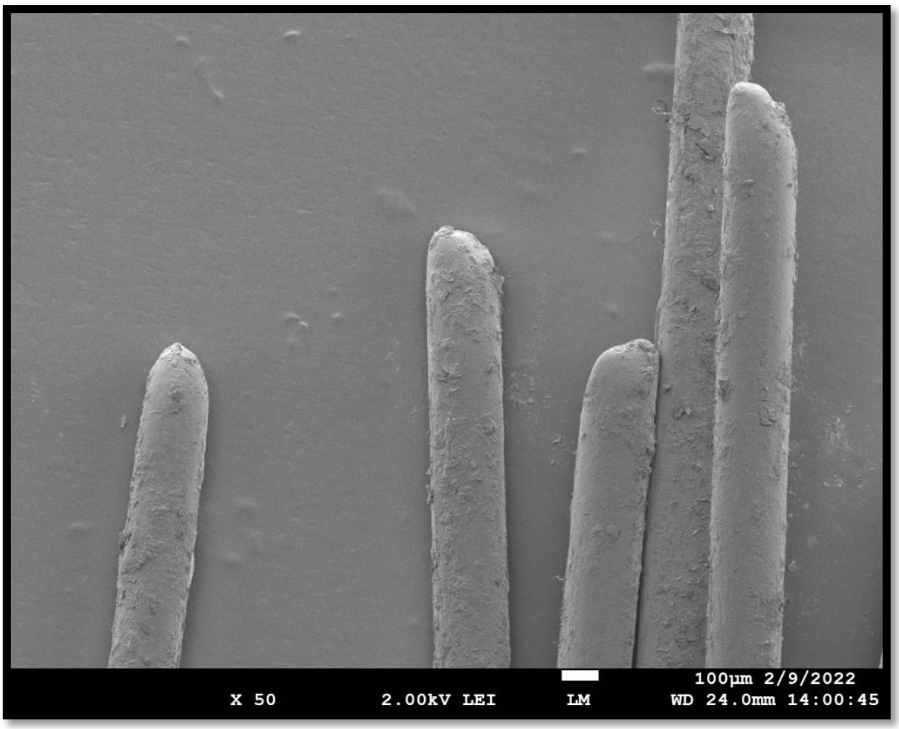


Figure16: Group A-Toothbrush Bristles at 50x Magnification.

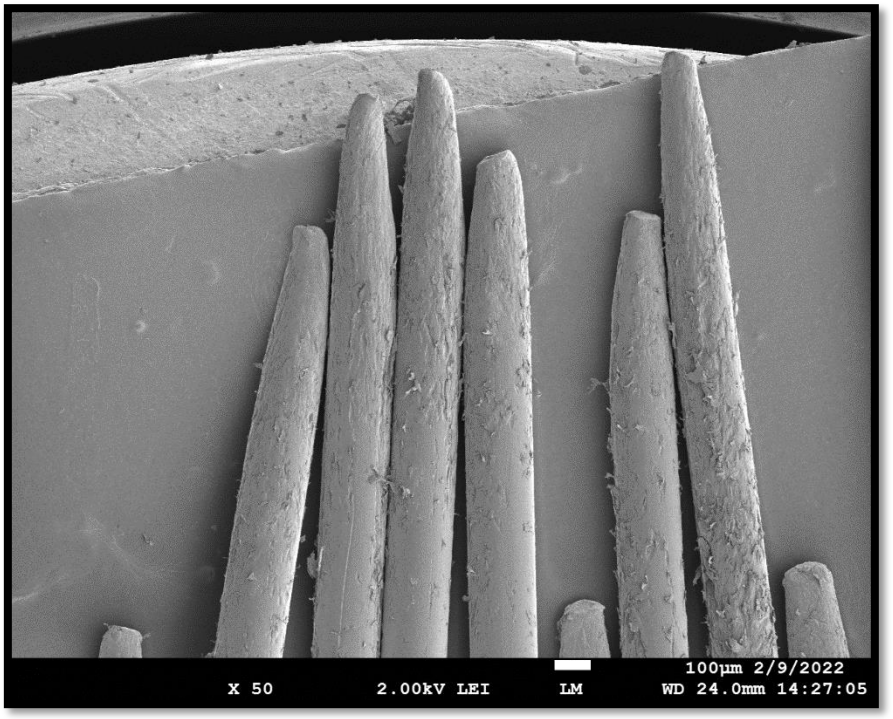


Figure 17: Group B- Toothbrush Bristles at 50x Magnification.

RESULTS

The study was carried out in the Department of Periodontology, BBDCODS,Lucknow and SEM part of the study was carried out at Birbal Sahani Institute of Palaeosciences Reasearch Institute ,Lucknow. The aim of the study was to assess the microbial load in between the bristle tufts on the head of the toothbrush. The Objective was to establish Colony Forming Units (CFU) of microorganisms and thickness of the bristles at baseline (the latter) after 3 and 4 months of toothbrush use.

The subjects were divided into two groups i.e.,

Group A -Toothbrush used for 3 months

Group B -Toothbrush used for 4 months

MICROBIOLOGICAL RESULT: -

Table 1: Descriptive analysis of CFU in group A

Minimum number of CFU calculated-	4
Maximum number of CFU calculated-	263
Mean value of CFU calculated-	71.32
SD -	69.64
Median -	50

In Group A it was observed that the minimum number of CFU calculated was 4 and the maximum was 263. The mean value of minimum and maximum CFUs calculated was 71.32. Since there was lot of variability in the data the median was calculated that was found to be 50.

Table 2: Descriptive analysis of CFU in group B

Minimum number of CFU calculated-	0
Maximum number of CFU calculated-	500
Mean value of CFU calculated-	165.28

SD- 189.27

Median- 92

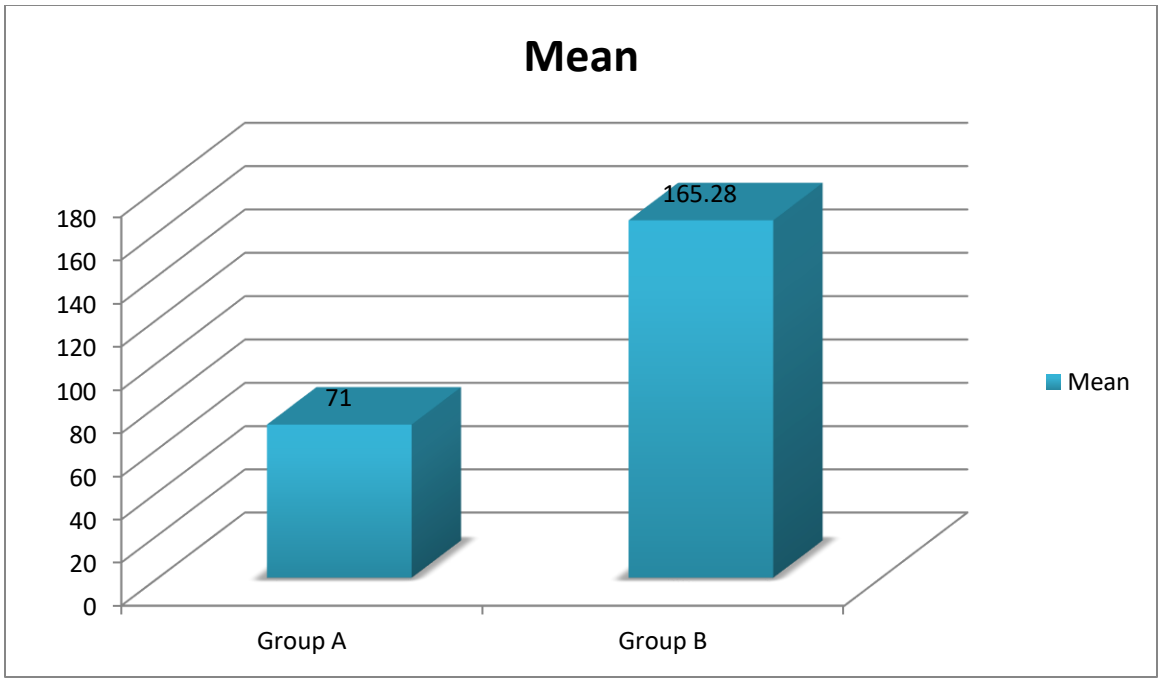
In Group B it was observed that the minimum number of CFU calculated was 0 and the maximum was 500. The mean value of minimum and maximum CFUs calculated was 80.10. Due to variability of data the median was calculated as 92.

Table 3: Comparison of CFU between group A and B

	Group A	Group B	t- test	P value
Mean CFU count	71.00	165.28	22.164	0.023
SD	68.69	189.27		
Median	47.00	92.00		

*: statistically significant

Table 3 and graph 1, shows the comparison of CFU between Group A and Group B. The results show that the mean CFU count of Group A is 71.00±68.69 which is less than Group B mean CFU count 165.28±189.27. The statistical analysis was done using t-test and was concluded that there is statistically significant difference between the two Groups ($p < 0.023$) with more CFU in Group B.



GRAPH 1: Depicts the mean CFU count in Group A and Group B .

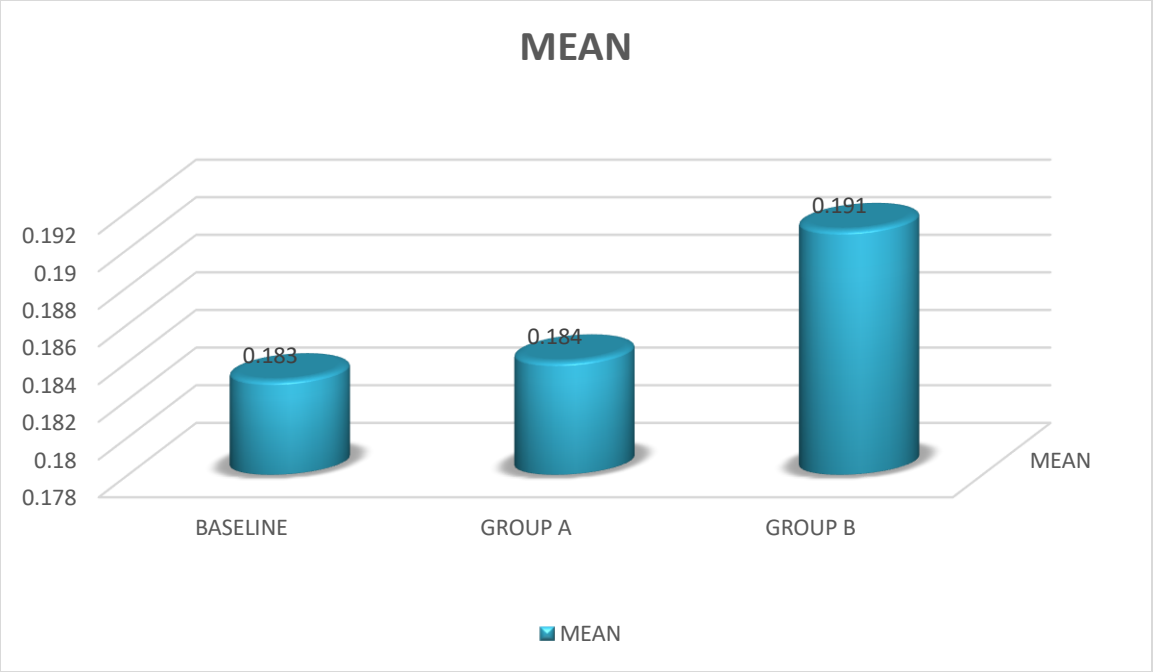
SEM RESULTS: -

Table 4:- Comparing The Change In Thickness Of The Bristles

BRISTLE THICKNESS	BASELINE	GROUP A	GROUP B	p value
MEAN	0.183	0.184	0.191	**0.003**
SD	0.000	0.000	0.005	

*Statistically significant

Table 4 and Graph 2 shows the comparison of mean bristle thickness at baseline(unused), Group A and Group B respectively and as observed that the result is statistically significant as the $p < 0.05$.



GRAPH 2 : Depicts the bristle thickness at baseline(unused), Group A and Group B.

Table 5: - Inter-Group Comparison of Bristle Thickness.

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	p-value
BASELINE	**GROUP A**	-0.001	0.002	0.884
	GROUP B	-0.008	0.002	**0.005**
GROUP A	**BASELINE**	0.001	0.002	0.884
	GROUP B	-0.007	0.002	**0.011**
GROUP B	**BASELINE**	0.008	0.002	**0.005**
	GROUP A	0.007	0.002	**0.011**

Table 5 shows the comparison of the Groups A and Group B with Baseline. When Baseline was compared to Group A the mean difference and p value was (-0.001) and (0.884) respectively. Since p value of this comparison >0.05, the findings are statistically not significant. When

baseline was compared to Group B, the mean difference and the p value was 0.008 and 0.005 respectively. Since p value of this comparison is <0.05, the findings are statistically significant. From the results it can be concluded that there was no significant difference between the baseline bristle thickness and Group A ($p>0.05$). Similarly, statistically significant difference was seen between the baseline thickness and Group B($p<0.05$). However, it was observed that there is no significant difference between Group A and Group B as the p value is greater than ($p>0.05$).

DISCUSSION

Bacteria are the primary etiological agent in gingival and periodontal diseases, and it is estimated that more than 500 different bacterial species are capable of colonizing the adult mouth. Due to the sheer increased knowledge of the importance of excellent oral health and dental professionals' emphasis on preventative procedures, the toothbrush has become the most frequent oral hygiene aid for promoting oral health and preventing dental illnesses.

Microorganisms found both inside and outside the oral cavity can contaminate toothbrushes[34,35]. According to studies it has been seen that toothbrushes can be a cause of recurrent infections of the mouth. Microorganisms adhere to the bristles & multiply exponentially within the bristles of used toothbrushes. Then through existing or fresh gingival abrasions they can transmit infections both locally & systemically. Since contamination & translocation of bacteria from one site to another is possible, an infected toothbrush, in all probabilities, can increase the microbial load and lead to periodontal infections or aggravate existing lesions. The area of the toothbrush where the tufts are fixed is particularly vulnerable to contamination.[36,37]Capillary movement can trap fluids and food particles into the gaps between tufts, there by contributing to bacterial development.

Glass discovered potentially harmful bacteria and viruses in toothbrushes from both healthy and sick individuals, including Staphylococcus aureus, E. coli, Pseudomonas, and herpes simplex virus. Glass also detected enough infected toothbrushes with the herpes simplex virus 1 to infect the patient[38]. Brushes used by individuals with oral disease quickly got infected, according to Bunetel et al. This study also discovered a link between repeated use and bacterial retention on toothbrushes, as well as the fact that a contaminated toothbrush can inoculate the oral cavity. Brushes were shown to be contaminated before usage in several trials[39,40]. Brushes are significantly polluted with typical use, according to Caudry et al.[41]. Taji and Rogers[42] and Glass[43] found substantial toothbrush contamination after use After use, 70 percent of the toothbrushes studied by Mehta et al. became severely contaminated with pathogenic microorganisms[44]. Except in cases where an oral antiseptic, such as mouthwash, was administered immediately prior to brushing. Studies have revealed that mostly aerobic species harbor the brush head. However, Muller et al found that Aa from patients with aggressive

periodontitis was still detectable on the toothbrushes after 24hours of use. According to Glass, microorganisms not only adhere to bristles and reproduce but also have the ability to cause both local and systemic diseases. Verran and Leahy-Gilmartin discovered that toothbrushes supported a wide range of bacteria, with varying levels of growth[45].

Several studies have also stressed[46,47,48] on the role of contaminated toothbrushes and its causation in systemic infections. In this regard, Brook and Gober suggested that contaminated toothbrushes contributed to the persistence of group A beta-hemolytic streptococci in the oropharynx and to the failure of penicillin therapy in some cases of pharyngotonsillitis[49]. In another study, Fischer pointed to a relationship between contaminated toothbrushes and pharyngitis[50]. Significant bacteremia has also been reported after tooth brushing, especially in patients with severe periodontitis[51,52].Therefore, a concern has been raised that the microbial load on toothbrushes might have a significant impact in periodontal patients under therapy[53].

In the present study, toothbrush head was assessed for the microbial contamination as it not only harbors micro-organisms but also provides a favorable environment for their growth. The nylon bristles are collected into bundles that are placed closely to each other. This placement not only eases the cleaning but also as mentioned previously fosters microbial growth. Microorganisms were assessed after 3 months (Group A) and after 4 months (Group B) of using the toothbrushes. The time duration was selected as 3 & 4 months since this is the most probable time toothbrushes are used for.

The study revealed micro-organisms to be present in the toothbrush head between the bristle tufts. Micro-organisms were assessed by counting the number of CFU formed on the agar plate. It was seen that in Group A the mean CFU count was 71.32; minimum being 04 and maximum being 263. In Group B, mean CFU was 165.28, minimum was 0 & maximum was 500. This 500 CFU was termed as lawn formation. There was a wide range (from minimum to maximum) of CFUs counted in both Group A & Group B. Also, in one of the samples no CFU was observed. This variation could be attributed to some aberration at the time of plaque collection, transportation, storage or even at the time of inoculation on the agar plate. Due to the dispersed CFU data in both the groups along the mean median was calculated for observation purposes.

When a comparision was made between Group A & Group B it was evident that the CFU in Group B was for higher & statistically significant shown in the p value (0.023). Hence it can be clearly stated that Group B had a greater number of CFUs in the bristles.

This result is in agreement with the study conducted by Willi-Eckhard Wetzel et al. in the year 2005[54]. Microorganisms were found to be present in all the toothbrushes except one which was used for 4 months. This may be due to failure in the collection process or failure in inoculation into agar plates.

Time necessary for colonization is contradictory varying from 1 to 30 days[55,56]. According to Cesco et al.[57], colonization of toothbrushes by mutans streptococci occurs in a short time period, since after a single toothbrushing, they found the development of the microorganism in 24% of the cases. Svanberg[58] reported the presence of microorganisms on toothbrushes after 3 days. In this study, colonization of bacteria was observed on bristles after 5 consecutive days of toothbrush use. Biofilm on the old toothbrush bristles was also observed despite the time of use and storage conditions. Storage conditions of toothbrushes are an important factor for bacterial survival. Dayoub et al.[59] and Meier et al.[60] reported that the number of microorganisms in the toothbrushes kept in aerated conditions was lower than in toothbrushes stored in plastic bags. Several authors have reported that bacterial contamination can be reduced by washing toothbrushes after use, and drying in aerated conditions[61,62,63,64]. Caudry et al.[65] reported that a wet environment increases bacterial growth and cross contamination. Therefore, as time increases between one toothbrushing and another, more microorganism development can occur in the toothbrushes stored in a wet/moisture environment[66].

The multiplication and increase in number of these microorganisms can pose a significant risk of dissemination for certain individuals at risk such as immunocompromised, graft, diabetic, cardiovascular disease and elderly patients[67,68,69,70,71,72,73,74,75,76,77,78], and may cause serious problems during pregnancy[79,80,81]. After use, 70 percent of the toothbrushes studied by Mehta et al. became severely contaminated with pathogenic microorganisms[82]. Except in cases where an oral antiseptic, such as mouthwash, was administered immediately prior to brushing, Taji and Rogers[83] and Glass[84] found substantial toothbrush contamination after use. Verran and Leahy-

Gilmartin discovered that toothbrushes supported a wide range of bacteria, with varying levels of growth[85].

Along with the microbial assessment of toothbrush head, SEM study of the bristles at their tips (the part that comes in contact with the tooth) was also done.

Scanning Electron Microscopy (SEM) is an instrument that produces a largely magnified image by using electrons instead of light to form an image. A beam of electrons is produced at the top of the microscope by an electron gun. The electron beam follows a vertical path through the microscope, which is held within a vacuum. The beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample. Once the beam hits the sample, electrons and X-rays are ejected from the sample. It is a popular method for studying and gaining information on the microstructure, morphology and composition of materials such as teeth, composites and ceramics. It has proven to be helpful to in dental research also. Images at high magnification 50x to 10000x and higher can be seen with SEM.

In the present study we assessed & compared the change in thickness of bristles at baseline (unused toothbrush), in Group A & in Group B under the SEM. The samples (bristle tips) were observed at 25x, 30x and 50x magnification.

The findings revealed that there was a statistically significant difference between the bristle tips at baseline and Group A, similarly there was a notable change in thickness of bristle tips between the unused toothbrush and Group B but minor or no difference was observed between two Groups i.e., Group A and Group B, that was statistically insignificant.

It was seen that the bristle tips at 50x magnification at baseline(unused) had uniform & smooth bristles the entire length & at the tip thay were gently rounded.In Group A though the bristles were more or less uniform in the entire length, they still exhibited some irregularities. However, the bristle tips still remained rounded and was clearly visible in the SEM photographs. In Group B, the irregularity of the bristles increased & was clearly visible in the SEM photographs. There were more number of microbial deposits observed as compared to Group A. In addition, it was seen that the end of the bristle tips had become pointed. A toothbrush with end-rounded filaments and a patient with correct toothbrushing habits are important for preventing tooth abrasions and gingival damage[86,87]. According to Breitenmoser et al. (1979), toothbrushes with sharp-ended filaments cause 30% more gingival recession. Even after 3 weeks of 2*À* daily

toothbrushing, which would presumably create some rounding, sharp filaments are still considered non-acceptable[88].

Toothbrushing to date is the most common & effective tool used to improve the oral health of an individual. Incorporated with the correct brushing habits, it is sufficient to maintain good oral & thereby systemic health. The bristles attached to the toothbrush head functions to disrupt the bacterial plaque along with the dentifrice. However, since the tufts are placed within extremely close proximity to each other, these can also lead to harboring and replication of bacteria from both within the oral cavity & from the environment. Various factors like different anchoring designs of tufts, also contribute to lodging & entrapment of the bacteria with in the bristles. Presence of moisture especially in colder environments act as a breeding ground for microorganisms. Since the time required for microorganisms to breed and replicate is very less, the need to replace the used toothbrushes becomes extremely important. To add to these dimensional & structural changes occurring at the bristle tips add to the improbability of oral health maintenance.

Hence, the need to change the toothbrushes every 3-4 months stands a scientific backing as proven in our above study.

CONCLUSION

When assessing the usage of a toothbrush, the microbial load and the wearing away of the bristles both have to be taken into consideration. The chances of reinfection and improper cleaning efficacy far outweigh the financial aspect of changing the toothbrush. We came to the following conclusions based on the microbiological analysis and SEM study:

1) Group B Toothbrushes, which were used for 4 months, had a higher number of Colony Forming Units than Group A Toothbrushes, which were used for 3 months.

2) Similarly, while comparing Group B Toothbrush Bristles to the Baseline Toothbrush Bristles, it was observed that there was wearing away of the bristle tips and increase in thickness of the bristle.

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Annexure I

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"A Study to assess Residual Microbial Contamination of toothbrush head"** submitted by **Dr Chetan Chaudhary** Post graduate student from the **Department of Periodontology** as part of MDS Curriculum for the academic year 2019-2022 with the accompanying proforma was reviewed by the Institutional Research Committee present on **19th December 2019** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

Annexure II

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIII[th] Institutional Ethics Sub-Committee

IEC Code: 26 **BBDCODS/03/2020**

Title of the Project: A Study to assess Residual Microbial Contamination of Toothbrush Head.

Principal Investigator: Dr. Chetan Chaudhary **Department:** Periodontology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Chetan Chaudhary,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 18[th] March, 2020.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Sahana S. Member	Reader, Department of Public Health Dentistry, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Lakshmi Bala 18/03/20

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

Forwarded by:

(Dr. B. Rajkumar)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road. Lucknow-226028

Annexure III

Approval Letter from Birbal Sahani Institute of Palaeosciences for SEM analysis

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: 91-0522-[illegible] 2740098
फैक्स/Fax : registrar@bsip.res.in
ई–मेल/E-mail : www.bsip.res.in / www.bsip.india.org
वेबसाइट/Website

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बीरबल साहनी पुराविज्ञान संस
BIRBAL SAHNI INSTITUTE OF PALAEOSCIE
53, विश्वविद्यालय
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लखनऊ-226
Lucknow-22
(भारत) (IN

(भारत सरकार के विज्ञान एवं प्रौद्योगिकी विभाग का एक स्वायत्तशासी संस्थान)
(AN AUTONOMOUS INSTITUTE UNDER DEPARTMENT OF SCIENCE & TECHNOLOGY, GOVERNMENT OF INDIA)

संख्या
No. .....

No.BSIP/IV/SA/2021-22 / C-4591

दिनांक
Dated .....

24.11.2021

Dr. Vandana A. Pant
Professor & Head
Department of Periodontology,
BBD College of Dentral Science
BBD Uniiversity
Lucknow.

Sub: *Utilization of SEM-EDS facility inBSIP*

Dear Sir/Madam

With reference to your letter dated Nil addressed to the Director, BSIP, requesting therein to provide SEM facility of the Institute. I am directed to inform you that the above facilities can be provided to Dr. Chetan Chaudhary MDS 3rd year Student of your department on the following rates:

Paid Research Scholars of Universities/ PG Colleges:

a) Sample processing and mounting	Rs.250 per sample
b) Gold Palladium coating	Rs.300 per stub
c) Sample examination and photography	Rs.300 per exposure

****Unpaid Research Scholars of Universities/ PG Colleges:**

a)Sample processing and mounting	Rs.250 per sample
b) Gold Palladium coating	Rs.150 per stub
c) Sample examination and photography	Rs.100 per exposure

EDAX charges of single spectrum	Rs.1000 per spectrum
CPD (Critical Point drying)	Rs.500 per sample

****The rates of Unpaid Students will only be applicable subject to production of Certificate from the concerned Head of the Department.**
Note: + 18% GST will be charged extra as per the Government of India Norms from time to time.

He may utilize the facility on or after 08.12.2021.
He may bring one CD-R of 700 MB for loading images

Yours faithfully,

24/11/2021

(Sandeep K. Shidhare)
Registrar

Copy to:
Convener, Electron Microscopy Committee, BSIP, Lucknow

Babu Banarasi Das College of Dental Sciences

(A constituent institution of Babu Banarasi Das University)

BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Participant Information Document (PID)

1. **Study title**

   A STUDY TO ASSESS RESIDUAL MICROBIAL CONTAMINATION OF TOOTHBRUSH HEAD.

2. **Invitation paragraph**

   You are being invited to take part in a research study, it is therefore important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully. Ask us for any clarifications or further information. Whether or not you wish to take part is your decision.

3. **What is the purpose of the study?**

   The purpose of the study is to assess residual microbial contamination of toothbrush head.

4. **Why have I been chosen?**

   You have been chosen for this study as you are fulfilling the required criteria for this study.

5. **Do I have to take part?**

   Your participation in the research is entirely voluntary. If you do, you will be given this information sheet to keep and will be asked to sign a consent form. During the study you still are free to withdraw at any time and without giving a reason.

6. **What will happen to me if I take part?**

   You will be one of 50 participants enrolled in the study. A toothbrush will be provided and you have to brush with it using modified bass method. It will be collected after 3 months or 4 months and will be assessed for the residual microbial contamination on the toothbrush head.

7. What do I have to do?

You do not have to change your regular lifestyles for the investigation of the study.

8. What is the procedure that is being tested?

The procedure will involve assessing the residual microbial contamination of toothbrush head.

9. What are the interventions for the study?

The participants will be randomly divided into 2 groups: - Group A-25 participants, they will use the toothbrush for 3 months. Group B-25 participants, they will use the toothbrush for 4 months. All will be taught the modified bass brushing technique and will be told to refrain from using any mouthwash during the duration of the study. They will be asked to rinse with water only. Upon completion of the time duration of both the groups, the toothbrushes will be collected & kept in saline medium under sterile conditions. Then the broth will be inoculated on culture plates to check for microbial colonies which will be examined under microscope.

10. What are the side effects of taking part?

There are no side effects on patients in this study.

11. What are the possible disadvantages and risks of taking part?

There are no risk or disadvantages of taking part in this study.

12. What are the possible benefits of taking part?

This study will help us to know the amount of residual microbial contamination on the toothbrush head.

13.What if new information becomes available?

If additional information becomes available during the course of the research you will be told about these and you are free to discuss it with your researcher, your researcher will tell you whether you want to continue in the study. If you decide to withdraw, your researcher will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form.

14. What happens when the research study stops?

If the study stops/finishes before the stipulated time, this will be explained to the patient/volunteer.

15. What if something goes wrong?

If any severe adverse event occurs, or something goes wrong during the study, the complaints will be handled by reporting to the institution (s), and Institutional ethical community.

16. Will my taking part in this study be kept confidential?

Yes, it will be kept confidential.

17. What will happen to the results of the research study?

The results of the study will be used to evaluate the residual microbial contamination on the toothbrush head. Your identity will be kept confidential in case of any report/publications.

18. Who is organizing the research?

This research study is organized by the academic institution (BBDCODS).

19. Will the results of the study be made available after study is over?

Yes.

20. Who has reviewed the study?

The study has been reviewed and approved by the Head of the Dept, and the IEC/IRC of the institution.

21. Contact for further information

Dr. CHETAN CHAUDHARY
Department of Periodontology
Babu Banarasi College of Dental Sciences.
Lucknow-227105
Mob- 7017422543

Dr.MONA SHARMA(HOD)
Department of periodontology
Babu Banarsi College of dental sciences
Lucknow-227105
Mob.-9984110444

Dr. VANDANA A PANT (PROFESSOR)
Department of Periodontology
Babu Banarasi College of Dental Sciences.
Lucknow-227105
Mob-9935957775
bbdcods.iec@gmail.com

बाबू बनारसी दास कॉलेज ऑफ डेंटल साइंसेज

(बाबू बनारसी दास विश्वविद्यालय का एक घटक संस्थान)

बीबीडी सिटी, फैजाबाद रोड, लखनऊ - 227105 (INDIA)

प्रतिभागी सूचना दस्तावेज (पीआईडी)

1. अध्ययन शीर्षक

TOOTHBRUSH HEAD की नियमित आवासीय परीक्षा के लिए एक अध्ययन।

2. निमंत्रण पैराग्राफ

आपको एक शोध अध्ययन में भाग लेने के लिए आमंत्रित किया जा रहा है, इसलिए आपके लिए यह समझना महत्वपूर्ण है कि अध्ययन क्यों किया जा रहा है और इसमें क्या शामिल होगा। कृपया निम्नलिखित जानकारी को ध्यान से पढ़ने के लिए समय निकालें। हमसे कोई स्पष्टीकरण या अधिक जानकारी के लिए पूछें। आप हिस्सा लेना चाहते हैं या नहीं यह आपका निर्णय है।

3. अध्ययन का उद्देश्य क्या है?

अध्ययन का उद्देश्य टूथब्रश सिर के अवशिष्ट माइक्रोबियल संदूषण का आकलन करना है।

4. मुझे क्यों चुना गया है?

आपको इस अध्ययन के लिए चुना गया है क्योंकि आप इस अध्ययन के लिए आवश्यक मानदंडों को पूरा कर रहे हैं।

5. क्या मुझे भाग लेना है?

अनुसंधान में आपकी भागीदारी पूरी तरह से स्वैच्छिक है। यदि आप करते हैं, तो आपको रखने के लिए यह सूचना पत्र दिया जाएगा और सहमति पत्र पर हस्ताक्षर करने के लिए कहा जाएगा। अध्ययन के दौरान आप बिना किसी कारण के किसी भी समय वापस लेने के लिए स्वतंत्र हैं।

6. अगर मैं भाग लूंगा तो मेरा क्या होगा?

आप अध्ययन में नामांकित 100 प्रतिभागियों में से एक होंगे। एक टूथब्रश प्रदान किया जाएगा और आपको इसे संशोधित बास विधि का उपयोग करके ब्रश करना होगा। यह 3 महीने या 4 महीने के बाद एकत्र किया जाएगा और टूथब्रश सिर पर अवशिष्ट माइक्रोबियल संदूषण के लिए मूल्यांकन किया जाएगा।

7. मुझे क्या करना है?

अध्ययन की जांच के लिए आपको अपनी नियमित जीवन शैली को बदलने की आवश्यकता नहीं है।

8. वह प्रक्रिया क्या है जिसका परीक्षण किया जा रहा है?

प्रक्रिया में टूथब्रश सिर के अवशिष्ट माइक्रोबियल संदूषण का आकलन करना शामिल होगा।

9. अध्ययन के लिए हस्तक्षेप क्या हैं?

प्रतिभागियों को यादृच्छिक रूप से 2 समूहों में विभाजित किया जाएगा: - समूह ए -25 प्रतिभागियों, वे 3 महीने के लिए टूथब्रश का उपयोग करेंगे। समूह बी -25 प्रतिभागियों, वे 4 महीने के लिए टूथब्रश का उपयोग करेंगे। सभी को संशोधित बास ब्रशिंग तकनीक सिखाई जाएगी और अध्ययन की अवधि के दौरान किसी भी माउथवॉश का उपयोग करने से बचना बताया जाएगा। उन्हें केवल पानी से कुल्ला करने के लिए कहा जाएगा। दोनों समूहों की समय अवधि पूरी होने पर, टूथब्रश को बाँझ परिस्थितियों में खारा माध्यम में एकत्र और रखा जाएगा। फिर शोरबा को माइक्रोबियल कालोनियों की जांच करने के लिए कल्चर प्लेटों पर टीका लगाया जाएगा जो माइक्रोस्कोप के तहत जांच की जाएगी।

10. भाग लेने के दुष्प्रभाव क्या हैं?

इस अध्ययन में रोगियों पर कोई दुष्प्रभाव नहीं हैं।

11. भाग लेने के संभावित नुकसान और जोखिम क्या हैं?

इस अध्ययन में भाग लेने का कोई जोखिम या नुकसान नहीं हैं।

12. भाग लेने के संभावित लाभ क्या हैं?

यह अध्ययन हमें टूथब्रश सिर पर अवशिष्ट माइक्रोबियल संदूषण की मात्रा जानने में मदद करेगा।

13 .क्या नई जानकारी उपलब्ध हो जाती है?

यदि अनुसंधान के दौरान अतिरिक्त जानकारी उपलब्ध हो जाती है, तो आपको इन के बारे में बताया जाएगा और आप अपने शोधकर्ता के साथ इस पर चर्चा करने के लिए स्वतंत्र हैं, आपका शोधकर्ता आपको बताएगा कि क्या आप अध्ययन जारी रखना चाहते हैं। यदि आप वापस लेने का निर्णय लेते हैं, तो आपका शोधकर्ता आपकी वापसी की व्यवस्था करेगा। यदि आप अध्ययन जारी रखने का निर्णय लेते हैं, तो आपको एक अद्यतन सहमति पत्र पर हस्ताक्षर करने के लिए कहा जा सकता है।

14. जब शोध अध्ययन रुक जाता है तो क्या होता है?

यदि अध्ययन निर्धारित समय से पहले बंद / खत्म हो जाता है, तो यह रोगी / स्वयंसेवक को समझाया जाएगा।

15. अगर कुछ गलत हो जाए तो क्या होगा?

यदि कोई गंभीर प्रतिकूल घटना होती है, या अध्ययन के दौरान कुछ गलत होता है, तो संस्थान (एस), और संस्थागत नैतिक समुदाय को रिपोर्ट करके शिकायतों को नियंत्रित किया जाएगा।

16. क्या इस अध्ययन में भाग लेने को गोपनीय रखा जाएगा?

हां, इसे गोपनीय रखा जाएगा।

17. शोध अध्ययन के परिणामों का क्या होगा?

अध्ययन के परिणामों का उपयोग टूथब्रश सिर पर अवशिष्ट माइक्रोबियल संदूषण के मूल्यांकन के लिए किया जाएगा। किसी भी रिपोर्ट / प्रकाशन के मामले में आपकी पहचान गोपनीय रखी जाएगी।

18. शोध का आयोजन कौन कर रहा है?

यह शोध अध्ययन शैक्षणिक संस्थान (BBDCODS) द्वारा आयोजित किया जाता है।

19. क्या अध्ययन के परिणाम अध्ययन के बाद उपलब्ध कराए जाएंगे?

हाँ।

20. अध्ययन की समीक्षा किसने की?

अध्ययन की समीक्षा की गई है और विभाग के प्रमुख, और आईईसी / आईआरसी द्वारा अनुमोदित किया गया है।

21. अधिक जानकारी के लिए संपर्क करें

डॉ। चैतन्य चौधरी

आवधिक विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ -227105

मोब- 7017422543

डॉ। मोना शर्मा (एचओडी)

पीरियडोंटोलॉजी विभाग

बाबू बनारसी दंत चिकित्सा महाविद्यालय

लखनऊ -227105

मोब.-9984110444

डॉ। वंदना ए पैनटी (प्रोफेसर)

आवधिक विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ -227105

मोब -9935957775

बी बी

DATA COLLECTION

MICROBIOLOGICAL RESULTS:-

Group A	CFU
1	152×10^6
2	263×10^6
3	36×10^6
4	71×10^6
5	11×10^6
6	241×10^6
7	148×10^6
8	19×10^6
9	13×10^6
10	45×10^6
11	36×10^6
12	55×10^6
13	13×10^6
14	21×10^6
15	4×10^6
16	91×10^6
17	42×10^6
18	7×10^6
19	31×10^6
20	47×10^6
21	56×10^6
22	98×10^6
23	122×10^6
24	61×10^6
25	92×10^6

Group B	CFU
1	0
2	7 X 10^6
3	23 X 10^6
4	10 X 10^6
5	44 X 10^6
6	263 X 10^6
7	Lawn formation
8	Lawn formation
9	5 X 10^6
10	84 X 10^6
11	4 X 10^6
12	12 X 10^6
13	3 X 10^6
14	121 X 10^6
15	Lawn formation
16	92 X 10^6
17	39 X 10^6
18	145X 10^6
19	174 X 10^6
20	Lawn formation
21	211 X 10^6
22	Lawn formation
23	12 X 10^6
24	291 X 10^6
25	92 X 10^6

SEM RESULTS: -

COMPARING THE CHANGE IN THICKNESS OF THE BRISTLES :-

NEW	3 MONTHS	4 MONTHS
0.183µm	1) 0.183µm	1) 0.187 µm
	2) 0.185µm	2) 0.186 µm
	3) 0.184µm	3) 0.2 µm
	4) 0.184µm	4) 0.190 µm
	5) 0.185µm	5) 0.194 µm
	6) 0.183	6) 0.186
	7) 0.183	7) 0.188
	8) 0.185	8) 0.191
	9) 0.184	9) 0.191

	10)	0.185	10)	0.187
	11)	0.183	11)	0.192
	12)	0.184	12)	0.194
	13)	0.183	13)	0.189
	14)	0.183	14)	0.193
	15)	0.185	15)	0.186
	16)	0.184	16)	0.193
	17)	0.183	17)	0.187
	18)	0.183	18)	0.196
	19)	0.185	19)	0.191
	20)	0.184	20)	0.189
	21)	0.183	21)	0.194
	22)	0.184	22)	0.192
	23)	0.183	22)	0.193
	24)	0.185	24)	0.190
	25)	0.183	25)	0.188

PATIENT PROFORMA

NAME: -

AGE: -

SEX: -

Toothbrush used for: 3 months / 4 months

CFU Findings: -

SEM Observations: -

Annexure X

Plagirism report

Curiginal

Document Information

Analyzed document	plaq.docx (D131931472)
Submitted	2022-03-29T16:24:00.0000000
Submitted by	
Submitter email	1180328003@bbdu.ac.in
Similarity	8%
Analysis address	1180328003.bbduni@analysis.urkund.com

Sources included in the report

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