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# Microbial Contamination of Metal Staples in Used Toothbrushes: A Potential Oral Health Risk

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**Abstract** **Background:** Toothbrushes are essential tools in maintaining oral hygiene; however, with prolonged use, they become reservoirs for microbial growth. While microbial contamination of bristles has been widely studied, little attention has been given to the metal staples that anchor the bristles potential hidden niches for biofilm development. **Objective:** To assess and compare microbial contamination and biofilm formation on the metal staples of toothbrushes used for 1, 3 and 6 months. **Methods:** Thirty manual toothbrushes were collected from users after 1 month (n = 10), 3 months (n = 10) and 6 months (n = 10) of regular use. Metal staple areas were swabbed under sterile conditions and microbial load was quantified using CFU counts on blood agar, MacConkey agar and Sabouraud agar. Microbial species were identified using standard biochemical methods. Biofilm structure and maturity were evaluated using confocal laser scanning microscopy (CLSM). **Results:** Microbial load significantly increased with duration of toothbrush use. Mean aerobic CFUs rose from $2.6\times10^4$ at 1 month to $13.7\times10^4$ at 6 months ($p<0.001$). CLSM revealed increased biofilm thickness (7.8-36.2 µm), greater EPS production and declining live/dead cell ratios with time. SEM confirmed dense, multilayered biofilm formation over the metal staples in 6 month samples. **Conclusion:** Metal staples in toothbrushes serve as significant microbial reservoirs, particularly with prolonged use. Replacement of toothbrushes within 1 to 3 months may help minimize microbial risks and maintain better oral health.

**Key Words** Toothbrush, Bacterial Contamination, Toothbrush Staple, Decontamination

## INTRODUCTION

Toothbrushes remain the most widely used and effective mechanical aid for maintaining oral hygiene, with millions of individuals worldwide relying on them daily to prevent dental plaque accumulation, caries and periodontal diseases. However, despite their crucial role in oral health, toothbrushes themselves are increasingly recognized as potential reservoirs for microbial contamination. Prolonged and repeated exposure to the oral cavity, combined with retention of moisture, toothpaste residues and environmental microorganisms, creates an environment highly conducive to microbial colonization and biofilm formation on toothbrush surfaces. Numerous studies have demonstrated the accumulation of diverse pathogenic and opportunistic microorganisms on toothbrush bristles, linking their persistence to risks of re-infection, cross-contamination and even systemic health implications in immunocompromised individuals [1].

While research has predominantly focused on the bristle portion of toothbrushes, relatively little attention has been directed toward the metallic staples used to anchor the bristles to the plastic head. These staples, commonly manufactured from stainless steel or aluminum alloys, are continuously exposed to moisture, saliva and fluctuating pH conditions. Over time, such exposure may initiate electrochemical reactions leading to corrosion, pitting and structural degradation of the metal surface. The resulting surface roughness, cracks and crevices provide ideal niches for microbial adhesion and growth, potentially transforming these inconspicuous components into hidden reservoirs of infection [2]. Furthermore, corrosion by-products, such as metal ions, may leach into the oral cavity and exacerbate tissue irritation or interact with microbial biofilms, altering their pathogenic potential [3].

Understanding the microbial contamination of metal staples in used toothbrushes is therefore critical, as these components may represent a neglected yet significant source of oral health risk. By investigating both the physicochemical changes in the metal staples and the associated microbial colonization, this study aims to

highlight the broader implications of staple degradation in relation to oral and systemic health [4]. Insights from such research can not only emphasize the importance of timely toothbrush replacement but may also stimulate the development of improved toothbrush designs and safer alternative materials that minimize corrosion and microbial adherence [5-8].

## METHOD

### Toothbrush Inclusion

- Standard manual toothbrushes with metal staples only
- Used twice daily by healthy adults, Stored under ventilated, non-humid conditions

### Sample Size

- Total = 30 toothbrushes
  - Group A (1 month): 10 used toothbrushes
  - Group B (3 months): 10 used toothbrushes
  - Group C (6 months): 10 used toothbrushes

### Microbial Sampling and Analysis

- Swabbing the metal staple area only with sterile saline-soaked swabs
- Culturing on:
  - Blood agar (aerobic count)
  - MacConkey agar (Gram-negative bacteria)
  - Sabouraud dextrose agar (fungi)
- CFU counts done using digital colony counter

**Identification via Gram Stain, Biochemical Tests**

**Biofilm Visualization (on 3 Toothbrushes per Group): Confocal Laser Scanning Microscopy (CLSM):** Metal staples were aseptically removed from used toothbrushes, rinsed with sterile Phosphate-Buffered Saline (PBS) and subjected to viability and biofilm staining. Samples were incubated with the LIVE/DEAD BacLight kit (SYTO 9 and Propidium Iodide) for 15 min in the dark to assess live and dead cells. For extracellular polysaccharides, Concanavalin A-Alexa Fluor 647 was used. Staples were mounted in PBS on glass slides with coverslips and imaged using a confocal laser scanning microscope (63× oil objective). Excitation/emission settings were 488/500-540 nm (SYTO 9), 561/590-650 nm (PI) and 633/650-700 nm (ConA). Z-stack images were acquired at 0.3 µm intervals and 3D reconstructions were generated using ImageJ/Fiji. Quantitative analysis of biofilm thickness, live/dead ratios and EPS distribution was performed with COMSTAT2. Unused staples served as negative controls and heat-killed staples were used as positive staining controls.

## RESULTS

The above image shows a mature, dense thick biofilm layer spreads across the surface. The intense red fluorescence corresponds to the areas with high CV retention, indicting substantial biofilm biomass. Lighter or bright zones are likely areas of high biofilm thickness or cell clusters with high extra cellular matrix density. The biofilm formed is robust, with heterogeneous density and regions of structural disruption.

The Figure 1 shows a mature, dense thick biofilm layer spreads across the surface. The intense red fluorescence corresponds to the areas with high CV retention, indicting substantial biofilm biomass. Lighter or bright zones are likely areas of high biofilm thickness or cell clusters with high extra cellular matrix density. The biofilm formed is robust, with heterogeneous density and regions of structural disruption.

Microbial load significantly increased with duration of toothbrush use. Mean aerobic CFUs rose from $2.6\times10^4$ at 1 month to $13.7\times10^4$ at 6 months ($p<0.001$). CLSM revealed increased biofilm thickness (7.8-36.2 µm), greater EPS production and declining live/dead cell ratios with time. SEM confirmed dense, multilayered biofilm formation over the metal staples in 6 month samples (Figure 2-4, Table 1-2).

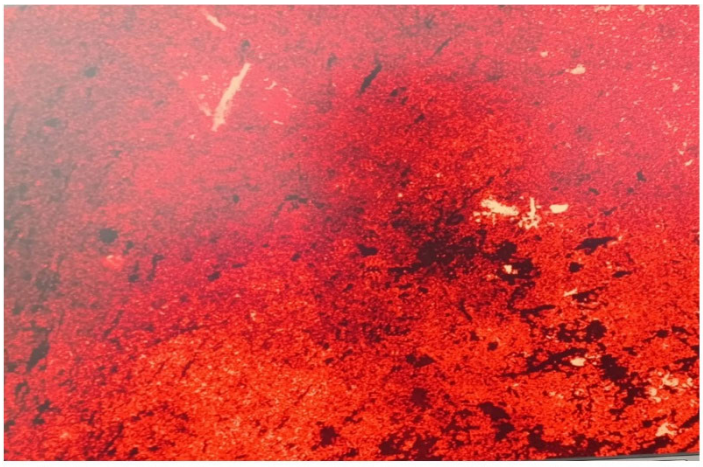

Figure 1: A Mature, Dense Thick Biofilm Layer Spreads Across the Surface

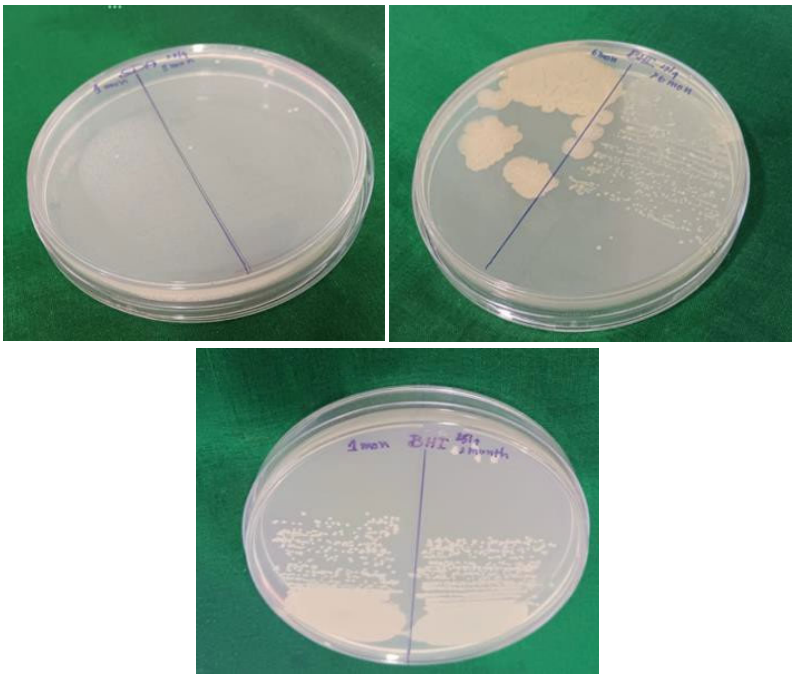

Figure 2(a-c): Microbial Load (a) 1 month, (b) 3 month and (c) 6 months

Table 1: Mean Microbial Load (CFU/mL)

| Group | Aerobic Bacteria (CFU/mL) | Gram-Negative Bacteria (CFU/mL) | Fungal Load (CFU/mL) |
|---|---|---|---|
| 1 Month | $2.6\times10^4\pm0.8\times10^4$ | $1.3\times10^4\pm0.6\times10^4$ | $0.2\times10^4\pm0.1\times10^4$ |
| 3 Months | $7.4\times10^4\pm1.1\times10^4$ | $4.3\times10^4\pm0.9\times10^4$ | $1.3\times10^4\pm0.4\times10^4$ |
| 6 Months | $13.7\times10^4\pm1.9\times10^4$ | $8.5\times10^4\pm1.5\times10^4$ | $3.0\times10^4\pm1.0\times10^4$ |

Table 2: CLSM Quantitative Analysis (n = 3 per group)

| Parameter | 1 Month | 3 Months | 6 Months |
|---|---|---|---|
| Biofilm Thickness (μm) | 7.8±1.2 | 19.4±2.3 | 36.2±3.8 |
| Live/Dead Cell Ratio | 3.2:1 | 1.7:1 | 0.8:1 |
| EPS Volume ($\mu m^3/\mu m^2$) | 0.8±0.1 | 2.3±0.3 | 4.5±0.5 |
| Surface Coverage (%) | 26.5±4.7 | 60.1±6.3 | 87.5±5.9 |

**Observational Interpretation**

The given graphical image represents that the given dental sample is supposed to show biofilm production and even distribution of biomass is observed through Confocal Laser Scanning Microscopy (CLSM).

**Red Background with Heterogeneous Texture**

Indicates a dense biofilm. The grainy texture represents cell clusters, ECM (extracellular matrix).

**Dark Patches/Regions**

They represent a low-density biomass region that has been less stained.

**Bright Spots**

They represent high fluorophore concentration, on cell aggregates localized biofilm matrix components via crystal violet stain.

## DISCUSSION

The present study highlights that metallic staples in toothbrushes, though small and often overlooked, can serve as significant reservoirs of microbial contamination. The findings are consistent with previous research on toothbrush bristles, which have long been recognized as potential sites for bacterial colonization [9]. However, the current focus on metal staples provides new insights into an underexplored aspect of toothbrush hygiene. The corrosion of staples, particularly when composed of stainless steel or aluminum alloys, introduces surface irregularities that facilitate microbial adhesion and biofilm formation [10]. This supports the hypothesis that corrosion not only weakens the physical stability of the toothbrush but also enhances microbial persistence [11].

Microbial growth on corroded staples may be attributed to the creation of microenvironments in the corroded regions that provide protection from mechanical cleaning and drying. Such protected niches allow pathogenic and opportunistic microorganisms to survive and potentially recolonize the oral cavity during repeated use of the toothbrush. Moreover, the leaching of metal ions such as iron, nickel or aluminum from corroded staples can alter the microbial ecology by promoting selective growth of metal-tolerant species [12]. This could exacerbate oral health risks, particularly in individuals with compromised immunity or pre-existing periodontal conditions.

The oral health implications of microbial contamination of metal staples extend beyond localized effects [13]. Persistent reintroduction of microorganisms from contaminated toothbrushes may contribute to recurrent infections, gingival inflammation and even systemic dissemination of pathogens, especially in susceptible populations [14].

Previous studies have shown associations between poor toothbrush hygiene and systemic conditions such as infective endocarditis and respiratory infections, underscoring the importance of understanding hidden contamination sources within toothbrush structures. Another important consideration is the frequency of toothbrush replacement. While dental associations generally recommend replacement every three months, evidence from this study suggests that microbial colonization of metal staples may begin earlier due to corrosion and environmental exposure. This raises the need to re-evaluate current guidelines and emphasize public awareness regarding hidden contamination risk [15]. Furthermore, manufacturers should consider alternative designs that eliminate metallic components or employ more resistant and biocompatible materials to mitigate both corrosion and microbial colonization.

Overall, the findings of this investigation highlight the dual role of metal staples as both structural elements and potential microbial reservoirs. Addressing this overlooked source of contamination is essential for improving oral hygiene practices and reducing oral and systemic health risks associated with toothbrush use. Future studies can focus on antimicrobial coatings for metal staples to inhibit microbial growth. The development of staple-free toothbrush designs or the use of biocompatible, non-metallic materials could reduce contamination risks. Research into user hygiene practices, storage conditions and replacement intervals may offer practical solutions to minimize microbial load [16]. Additionally, standardized microbial testing of used toothbrush components can support safer product design and health guidelines.

## CONCLUSIONS

Metal staples in toothbrushes showed a progressive accumulation of microbial load and biofilm architecture with increasing use. By 6 months, staple regions were almost fully covered with mature, organized biofilm, including dead cells and EPS. These findings support replacing toothbrushes within 1-3 months to reduce microbial risk.

## Acknowledgement

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## Ethical Approval

The study protocol was reviewed and approved by the saveetha Dental college and Hospital IHEC/SDC/PHD/PROSTHO-2426/25/TH-007 Written informed consent to participate was obtained from all participants prior to enrollment in the study. For participants under 18 years of age, consent was obtained from a parent or legal guardian.

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