

PCR-BASED APPROACH TO ASSESS THE RETAINED MICROFLORA AND THE PRESENCE OF HUMAN DNA ON USED TOOTHBRUSHES

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Toothbrushes, the most common article used to maintain oral health, are constantly subjected to contamination with the oral, environmental microflora, oral fluids and epithelial cells during storage and usage. The favourable atmosphere created by the residual moisture and food debris facilitates microbial growth, leading to biofilm formation. Retained microflora on toothbrushes potentially influences both oral and systemic health. The present study was designed to assess retained microflora and the presence of human DNA on used toothbrushes by a group of Sri Lankans. Used toothbrushes were randomly collected from 20 volunteers (male=10, female=10) within 24 hours of their last use. Total DNA from the brush head and the bristles were extracted using modified phenol: chloroform protocol. The presence of human DNA was assessed using the B2M primer pair, while the clinically important bacteria *Porphyromonas gingivalis*, *Helicobacter pylori*, *Staphylococcus aureus*, and *Escherichia coli* were detected using specific 16S rRNA primers. DNA from the reference isolates of each tested bacteria and DNA from human blood were used as controls. To visualize the biofilm, a toothbrush bristle was coated with gold under vacuum for 40 mins and then examined with SEM at 20 kV. PCR demonstrated that the used toothbrushes harbour *P. gingivalis* (2/15), *H. pylori* (1/15), *S. aureus* (2/15), and *E. coli* (4/15). Human DNA was confirmed in (3/15) toothbrushes. SEM revealed bacteria attachment and biofilm formation on the used brush bristles compared to the unused ones. Hence, toothbrushes are a potential reservoir for clinically important bacterial species and can be useful sources of genomic DNA for forensic application. SEM suggests that the retained bacteria exist in biofilm form, and it is advisable to maintain toothbrush hygiene to gain satisfactory oral and general health, especially regarding immunocompromised individuals.

Keywords: 16S rRNA, Bacteria, Microbial biofilms, Oral health, Scanning electron micrographs