

# Investigating The Efficacy Of Denture Biofilm Cleaning Methods Using High-Resolution XCT

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## Aims

The use of removable dental prostheses is increasingly common, particularly with an ageing global population, as they help to maintain normal oral functionality and aesthetic appearance for the wearer. To avoid complications such as denture stomatitis, caused by biofilm and bioburden accumulation, specialist cleaning methods are required.

To demonstrate biofilm cleaning efficacy, conventional techniques such as viable cell counts and scanning electron microscopy (SEM) are routinely used<sup>1-3</sup>. However, these methods suffer from a number of drawbacks, particularly in their sensitivity due to issues around cell recovery from the denture (in context of viable cell counts) and in the small sample size (in context of SEM) in relation to the size of the dental prosthesis. Additionally, the complex topographical nature of dental prostheses means that evaluating the efficacy of cleaning from hard-to-reach areas is beyond the capability of many conventional methods.

The aim of this study, was to develop an *in vitro* method to assess biofilm removal from denture material, at meaningful scale and resolution using high-resolution X-ray computer tomography (XCT).

## Methods

- Sections of denture containing four units/teeth from the lower posterior (Figure 1) were cut, brushed with a standard toothbrush under hot running water to remove gross debris and sterilised by immersion in 100 % EtOH for 20 min.
- The denture was then immersed in tryptic soy broth (TSB) containing  $10^6$  CFU/ml *K. pneumoniae* ATCC 700831 and incubated for 96 h at 37 °C, with fresh media changes every 24 h.
- After 96 h biofilm growth, the denture sections were removed and stained with 1 % phosphotungstic acid (PTA) in 70 % EtOH for 1 h followed by a 5 s rinse in Hanks balanced salt solution to increase the contrast of the biofilm.
- Denture sections (n=4/treatment) were either treated with a new ultrasonic denture cleaning bath (Dental Labo) in combination with a denture cleanser (Polident Neo) tablet for 5 min, according to consumer use instructions, or brushed with a standard toothbrush to comparatively evaluate mechanical removal. For the brushing, a new model was developed that permitted the automatic control of the brushing time (6 s/tooth), brushing orientation and load applied on the teeth (50 N).
- High resolution XCT imaging was performed at 70 V and 6 W using 1601 projections and 1.2 s exposure time and reaching 37  $\mu$ m voxel size (Xradia 520 Versa, Zeiss).



Figure 1. Photograph of denture used in study

## Results

High resolution XCT imaging: a useful tool for studying denture cleaning efficacy

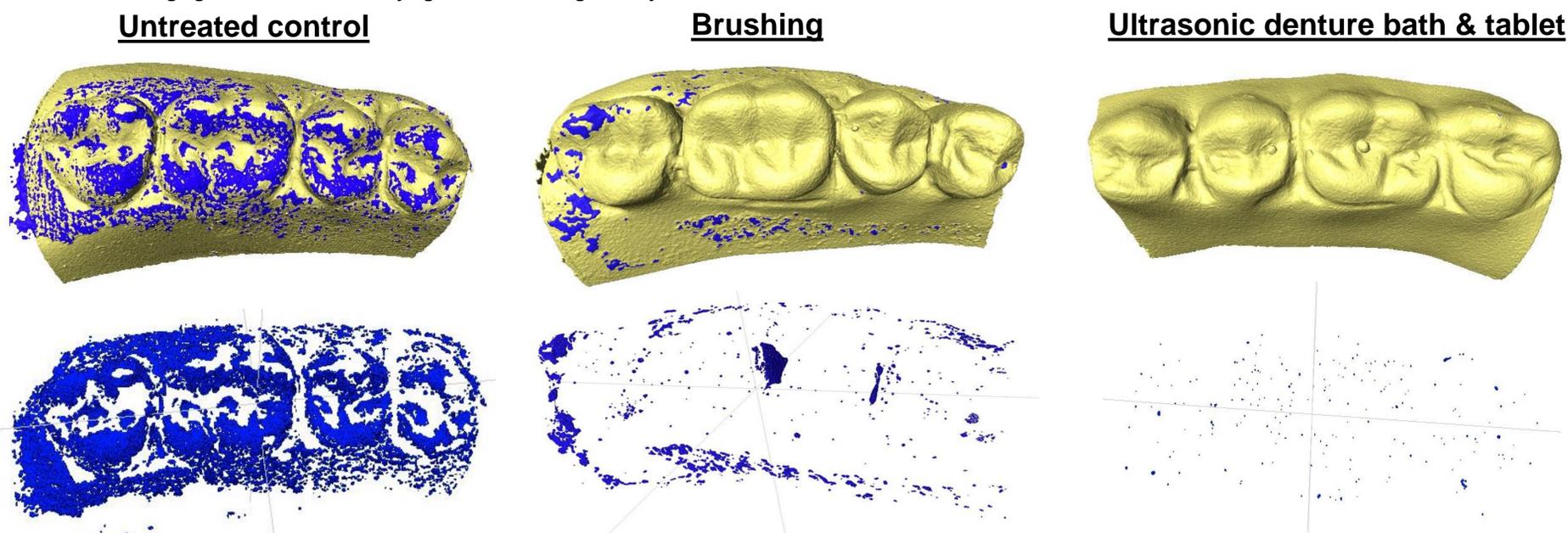


Figure 2. Representative High Resolution XCT images showing the effect of brushing and the ultrasonic denture bath on biofilm removal from dentures. Denture material is identified in yellow with the biofilm shown in blue. Images demonstrate that brushing is able to remove a significant amount of biofilm from the denture but that biofilm regularly remains in the hard to reach places of the denture. This is in comparison to the ultrasonic denture bath, combined with denture tablet, which is able to remove biofilm from all areas of the denture, including the hard to reach places such as the interproximal (IP) spaces.

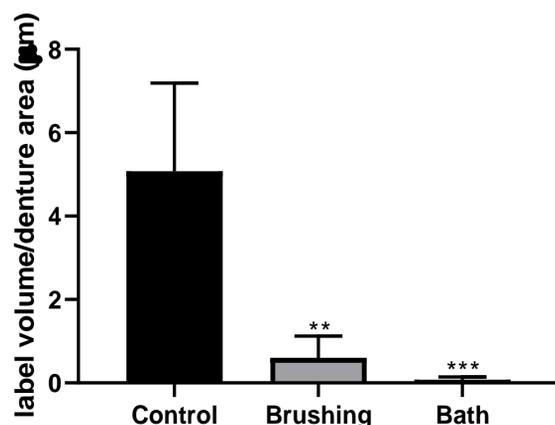


Figure 3. Data quantified from XCT scans showing volume of biofilm per surface area of denture. Ultrasonic denture bath reduces biofilm by 4.9907  $\mu$ m (98.26 %) from the control, relative to brushing (4.4777  $\mu$ m and 88.16 %). Data are reported as mean  $\pm$  SD (n=4). Statistics are one-way ANOVA which returned  $p=0.0006$ . Tukey's multicomparisons test results are indicated in the graph. \*\* $p<0.01$ , \*\*\* $p<0.001$  compared to control. There was no statistical difference between brushing and bath ( $p=0.8284$ ).

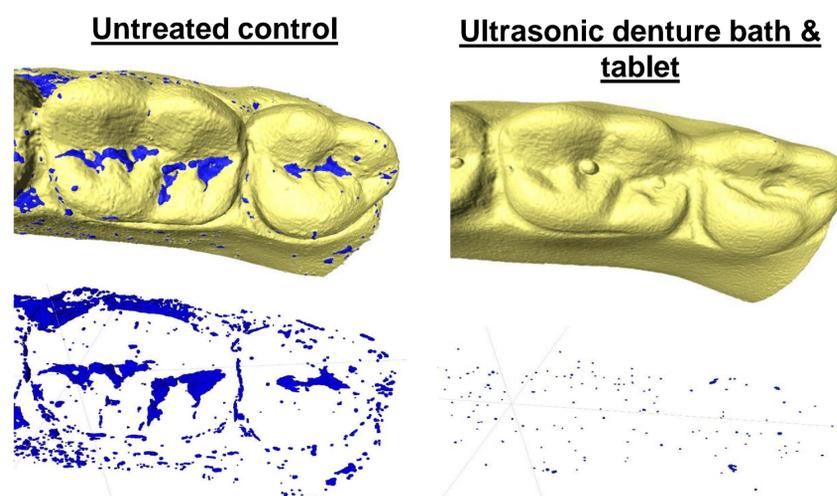


Figure 4. Representative XCT images showing in higher magnification the pits and fissures of the teeth and IP space demonstrating the efficacy of the ultrasonic denture bath & tablet combination at removing biofilm from hard to reach places.

## Conclusions

- High resolution XCT is an extremely useful tool to evaluate the efficacy of denture cleaning methods at meaningful scale and resolution, with the ability to image whole dentures or partial units, as well as the ability to focus on hard to reach areas at high magnification
- Both brushing and the ultrasonic denture bath combined with tablet treatments were capable of removing significant biofilm from the dentures
- However, brushing was less capable of removing biofilm from hard to reach areas of the denture, particularly the IP space, in contrast to the ultrasonic denture bath & tablet which showed good removal across all areas of the denture.

## References

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## Acknowledgements

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