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Imaging and analysis of individual cavitation microbubbles around dental ultrasonic scalers

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ABSTRACT

Cavitation is a potentially effective and less damaging method of removing biofilm from biomaterial surfaces. The aim of this study is to characterise individual microbubbles around ultrasonic scaler tips using high speed imaging and image processing. This information will provide improved understanding on the disruption of dental biofilm and give insights into how the instruments can be optimised for ultrasonic cleaning. Individual cavitation microbubbles around ultrasonic scalers were analysed using high speed recordings up to a million frames per second with image processing of the bubble movement. The radius and rate of bubble growth together with the collapse was calculated by tracking multiple points on bubbles over time. The tracking method to determine bubble speed demonstrated good inter-rater reliability (intra class correlation coefficient: 0.993) and can therefore be a useful method to apply in future studies. The bubble speed increased over its oscillation cycle and a maximum of 27 ms^{-1} was recorded during the collapse phase. The maximum bubble radii ranged from 40 to 80 μm. Bubble growth was observed when the ultrasonic scaler tip receded from an area and similarly bubble collapse was observed when the tip moved towards an area, corresponding to locations of low pressure around the scaler tip. Previous work shows that this cavitation is involved in biofilm removal. Future experimental work can be based on these findings by using the protocols developed to experimentally analyse cavitation around various clinical instruments and comparing with theoretical calculations. This will help to determine the main cleaning mechanisms of cavitation and how clinical instruments such as ultrasonic scalers can be optimised.

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1. Introduction

The dynamics of individual cavitation bubbles are of interest to many scientific disciplines including hydraulics (propellers, turbines and pumps), ultrasound cleaning and biomedical engineering. The aim is to understand the mechanisms underlying the surface cleaning, erosion and sonoporation effects [1]. The characteristics of microbubbles around dental ultrasonic scalers are directly related to cavitation cleaning behaviour but the exact mechanisms are not fully understood [2]. Current methods of dental plaque biofilm removal are predominantly mechanical and are not effective in removing it from irregular surfaces in the mouth. Cavitation occurring around dental ultrasonic scalers may be a more efficient and less damaging technique. Previous work has failed to quantify the cavitation bubble dynamics around ultrasonic scalers and its effects. Understanding the cavitation bubble dynamics could help to provide insights into how the cavitation can clean biofilms. This will enable manufacturers to develop instruments that optimise the cavitation cleaning effects [3].

High speed imaging of bubbles combined with image analysis is a non-intrusive method of experimentally investigating cavitation, and can provide detailed information on bubble structure without interfering with their dynamic activity [4–7]. A considerable amount of literature has been published on the mathematical modelling of individual cavitation bubble behaviour to understand how they clean or erode surfaces [8–16]. Chahine et al. simulated bubbles near different boundaries (rigid, elastic and free surface) to measure the pressure driving the bubble and found that the distance between the bubble and the surface to be cleaned influenced the cleaning [17]. Theoretical calculations have also been combined with experimental validation using high speed imaging [10,18]. These studies have calculated the evolution of the bubble

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radius over time and modelled the diffusion inside the liquid for different applications such as drug delivery and erosion. However no such research has been conducted on cavitation bubbles from ultrasonic scalers.

A range of phenomena have been identified which occur during the collapse of cavitation bubbles and may contribute to their cleaning effect. If a cavitation bubble collapses near a boundary, such as a wall or another bubble, it will form a high velocity micro liquid jet which penetrates through the centre of the bubble and impacts the opposite surface with large local stresses [8]. The re-entrant jet penetrates the bubble at a higher velocity than the rest of the collapsing bubble surface, impacting the opposite surface at speeds of over 100 m/s and a pressure of ~400 MPa [19]. It is believed that the jet and resulting shock wave imposes a localised high hydrodynamic load on the solid surface nearby, removing the biofilm off the surface [1]. In terms of damage from erosion, Phillip and Lauterborn identified that the jet only has an effect when the bubble is very near to or touching the surface, and that the main mechanism is the formation of a vortex ring bubble (a torus shaped bubble rotating poloidally) which collapses producing high pressure pulses [10,19]. This may also contribute to ultrasonic cleaning but the exact bubble dynamics which lead to biofilm removal remain to be elucidated [1]. Cavitation bubbles can also lyse cellular membranes and could kill bacterial biofilm as well as disrupting it, but the cellular pathways which occur in bacteria when cavitation is applied have not yet been identified [1,20].

Quantitative research on cavitation around endodontic files has been done using high speed particle imaging velocimetry and computational fluid dynamics to show the fluid flow and acoustic microstreaming occurring around the files [21,22]. In terms of high speed imaging to study cavitation around dental instruments, individual microbubbles have been imaged around endodontic files and lasers [23–26]. Peeters et al. showed how air trapped in a root canal can be released and Matsumoto et al. showed that more cavitation occurred in an enclosed root canal model compared to in free space. Macedo et al. used high speed imaging to demonstrate cavitation occurring at the end of endodontic files, and qualitatively observed how the cavitation cloud changed with different irrigant solutions [27]. However these studies have not performed image analysis of individual cavitation bubbles due to the limited temporal and spatial resolution. In addition, very little is known about the characteristics of individual cavitation bubbles around ultrasonic scalers. Bubble dynamics around ultrasonic scaler tips have not yet been studied using high speed imaging combined with image processing. Observing bubble phenomena will help to determine the timescales of growth and collapse, how this is affected by scaler power settings and where nucleation sites are located. This will contribute to discovering how cavitation microbubbles cause biofilm disruption.

The aim of this study is to characterise individual microbubbles around ultrasonic scaler tips using high speed imaging and image processing. Specifically, we aim to observe cavitation bubble phenomena and calculate bubble speed and radius during the growth and collapse phases.

2. Materials and methods

2.1. High speed imaging

An ultrasonic scaler (P5 Newtron, Satelec, Acteon, France) was imaged with tip 10P at various power settings (Power 5, 7, 10, 15, 20 (maximum)) using high speed cameras. It should be noted that the power control dial of the ultrasonic scaler is not a reproducible measure of power. The power output of the ultrasonic scaler cannot be measured accurately due to the tip shape, however Walmsley et al. have shown that the displacement amplitude of the tip is that main factor which has to be controlled [28,29]. The displacement amplitude of the tip is given in Vyas et al [30].

A Photron SA1.1 high speed camera (Photron, San Diego, CA, USA) was used to image bubbles at 250 k frames per second (fps) or 500 kfps. The camera was attached to a zoom lens (Monozoom 7, Leica Microsystems UK Ltd) to obtain a resolution of 5.6 μm/pixel. The size of each pixel was calculated from measurements of a 2 mm graticule with 10 μm markings. More information about the experimental setup using the Photron camera is given in Vyas et al. [30].

To give more details about the bubble collapse phase, an ultra-fast high speed camera (HPV1, Shimadzu Corporation, Japan) was used to image cavitation microbubbles around tip 10P at 1,000,000 fps. The camera was attached to a zoom lens (Monozoom 7, Leica Microsystems UK Ltd) to obtain a resolution of 6.7 μm/pixel (Fig. 1). The size of each pixel was calculated from measurements of a 2 mm graticule with 10 μm markings. The difference in resolution between the two imaging systems is due to differences in their focal lengths. Illumination was provided by two strobe lights which were synchronised with the camera using a flash light controller, delay generator and trigger switch. The scaler was positioned using a translation stage (PT3, Thorlabs, USA). The scaler tip was imaged in a custom-made glass container with a total volume of 10 ml. The container was made by cutting glass microscope slides to 2.7 × 2.7 mm and attaching 5 squares to each other using glass adhesive (Loctite, USA) to create an open cube. The scaler tip was submerged in the container in 10 ml reverse osmosis water at 20.5 °C.

2.2. Image analysis

All image analysis was done using Fiji (distribution of the ImageJ software, US National Institutes of Health, Bethesda, Maryland, USA) [31]. Data graphing was done using SigmaPlot 12.3 (Systat Software, USA) and statistical analysis was performed using SPSS (IBM, USA). The image analysis steps described below are also summarised in Supplementary Figure S1.

Image sequences where individual bubbles were seen to grow and collapse completely within the imaging field of view were used to extract the outline of the bubbles for further analysis. These images were first cropped and segmented using the Trainable Weka Segmentation Plugin [32]. Some parts of the background were falsely segmented and were eliminated by removing objects smaller than 4 pixels using the Analyse particles plugin. Objects touching the edge of the image were also removed (using the 'exclude on edges' feature in the Analyse particles plugin) to remove other bubbles which were partially in the field of view. The outline of the bubble was then created using the 'outline' function in the Binary menu of Fiji. For the cases where there were multiple individual bubbles in the images, a watershed segmentation was performed to separate bubbles in the image which were touching each other before creating an outline of the binary bubble shapes. These steps were completed for all time points during a bubble's growth and collapse.

The x-y coordinates of the binary outline of two bubbles were saved for an image sequence with an inter-frame time of 2 μs and plotted as a 3D graph to visualise the bubble localisation during the different stages. 3D visualisation (with the 3rd dimension being time) was also done using the 3D viewer in Fiji for multiple individual bubbles observed simultaneously. To aid in visualisation through time, the image sequence was colour coded to show the different bubble behaviour at different time points.

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