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Improved Biofilm Removal using Cavitation from a Dental Ultrasonic Scaler Vibrating in Carbonated Water

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Abstract

The use of cavitation for improving biofilm cleaning is of great interest. There is no system at present that removes the biofilm from medical implants effectively and specifically from dental implants. Cavitation generated by a vibrating dental ultrasonic scaler tip can clean biomaterials such as dental implants. However, the cleaning process must be significantly accelerated for clinical applications. In this study we investigated whether the cavitation could be increased, by operating the scaler in carbonated water with different CO_2 concentrations. The cavitation around an ultrasonic scaler tip was recorded with high speed imaging. Image analysis was used to calculate the area of cavitation. Bacterial biofilm was grown on surfaces and its removal was imaged with a high speed camera using the ultrasonic scaler in still and carbonated water. Cavitation increases significantly with increasing carbonation. Cavitation also started earlier around the tips when they were in carbonated water compared to non-carbonated water. Significantly more biofilm was removed when the scaler was operated in carbonated water. Our results suggest that using carbonated water could significantly increase and accelerate cavitation around ultrasonic scalers in a clinical situation and thus improve biofilm removal from dental implants and other biomaterials.

Introduction

Cavitation is the formation and the subsequent dynamics of a cloud of bubbles in a liquid or in a tissue, typically induced by ultrasound or high-speed flows. Cavitation bubbles are vacuum or filled with vapour, gas or a mixture [1]. When driven into oscillation by ultrasound, such bubbles are capable of yielding microstreaming, shock waves, high-speed jets and high heating, which are detrimental in numerous applications. The energy released during bubble implosion is also used in many industries for cleaning [2-4]. Cavitation for removing biofilms from surfaces has potential as an effective cleaning technique. Cavitation bubbles can reach small crevices so that they can remove bacterial biofilm more efficiently from biomaterials with microscopically roughened surfaces such as dental implants [5-7]. The use of titanium metallic implants is well established in dentistry and their use is growing [8]. These implants are designed with specialised surface treatments for bonding to bone [9]. Dental plaque biofilm formation on implant surfaces can lead to gum disease, which can cause loss of supporting bone and subsequent implant failure. Therefore it is important to effectively remove biofilm to prevent and treat peri-implant mucositis and peri-implantitis.[10, 11]. Current methods of cleaning the biofilm are either not effective at the microscopic level or they involve using metal hand or ultrasonic instruments that lead to potentially damaged surface which could cause problems during re-osseointegration [12-14]. There is no system at present that removes the biofilm from implants effectively and safely [15, 16].

Ultrasonic scalers are used in dentistry to remove mineralised plaque from teeth by lightly scraping a vibrating metal tip across the surface of teeth [17]. The metal tip vibrates at ultrasonic frequencies and can create cavitation bubbles in the cooling water flowing over the tip[18]. Enhancing the cavitation occurring around ultrasonic scalers could lead to quicker biofilm cleaning. Our recent *in vitro* experiments have shown that cavitation from ultrasonic scalers is able to remove biofilm from dental implant surfaces [19]. This suggests the possibility of a contact-free approach to cleaning dental implant surfaces, without the metal tip contacting the teeth or implants, causing less damage. However, significant cleaning occurred only after the tip of the scaler was held 1 mm away from the biofilm for 60 s [19]. This is not practical for use clinically where rapid cleaning is required (i.e. a few seconds).

One method of increasing the amount of cavitation bubbles is to increase the gas inside the fluid [20] to facilitate cavitation inception. This has been done in previous studies by using microbubbles in water generated by adding air or oxygen microbubbles [21-23] and this has enhanced ultrasonic cleaning. However this has not been investigated for biofilm removal applications. In this study we tested if cavitation around ultrasonic scalers can increase using carbonated water. Cavitation forms vapour cavities in a liquid when the liquid evaporates. These cavities are formed at a place in the liquid where the pressure is relatively low and the liquidised vapour becomes supersaturated. Cavitation can be enhanced by carbonated water because much more liquidised CO_2 is evaporated than liquidised vapour subject to the same pressure decrement [24]. The cleaning effect due to the pressure waves generated by an oscillating ultrasonic scaler is proportional to the gradient of the pressure wave, which is at order of $O(p_A/\lambda)$, where p_A and λ are the amplitude and wavelength of pressure waves. When cavitation happens, the gradient of pressure and shear stress is at the order of $O(p_A/R)$, where R is the bubble radius. Since microbubble radii are often much smaller than acoustic wavelength, cavitation enhances the cleaning significantly. Additionally, carbonated water is inexpensive, safe for patient use [25] and easily produced, therefore allowing rapid adoption of this method into clinical practice. We used high speed imaging and image analysis to investigate how carbonation changes the cavitation occurring around a dental ultrasonic scaler tip, and how it affects biofilm removal.

Methods

Carbonated water production

Reverse osmosis (RO) water at 14°C was carbonated using an automatic carbonator (SodaStream Power, SodaStream International Ltd, Israel). Three levels of carbonation are possible using this instrument, which will be referred to as low, medium and high (Table 1). 840 ml of RO water was carbonated for each setting. Experiments were done as soon as the water was carbonated to minimise loss of CO_2. The CO2 at each carbonation setting was approximated by filling balloons with the CO2 gas dispensed by the gas jet nozzle of the carbonator without water. The mass of the balloon before filling was subtracted from the

mass after filling, measured on a precision balance. This was repeated 3 times to calculate the mean and standard deviation, and converted to g/L for each carbonation setting.

Carbonation Setting	Time to carbonate (s)	CO_2 (g/L)
Low	4.72±0.18	0.43±0.02
Medium	5.94±0.20	0.52±0.02
High	8.97±0.11	0.81±0.08

Table 1 Details of the carbonated water produced using the SodaStream Power automatic carbonator, using RO water

High Speed Imaging of cavitation

A P5 Newtron XS scaler (29 kHz operating frequency) (Satelec, Acteon, France) was used in conjunction with Tip 10P (Figure 1a-c) operating at the maximum power setting. The ultrasonic scaler tip was immersed in a glass water tank (180 ml) (Figure 1) and its position was fixed by attaching it to a *XYZ* translation stage (PT3, Thorlabs Inc, NJ, USA) and a high-precision rotation mount (PRO1/M, Thorlabs Inc, NJ, USA). The axial rotation of the scaler tip was also maintained during each experiment. The scaler was illuminated using an LED cold light source (Hayashi HDF7010, Japan) in bright field mode. The scaler was positioned at the same location inside the image frame for all repeats to ensure reproducibility.

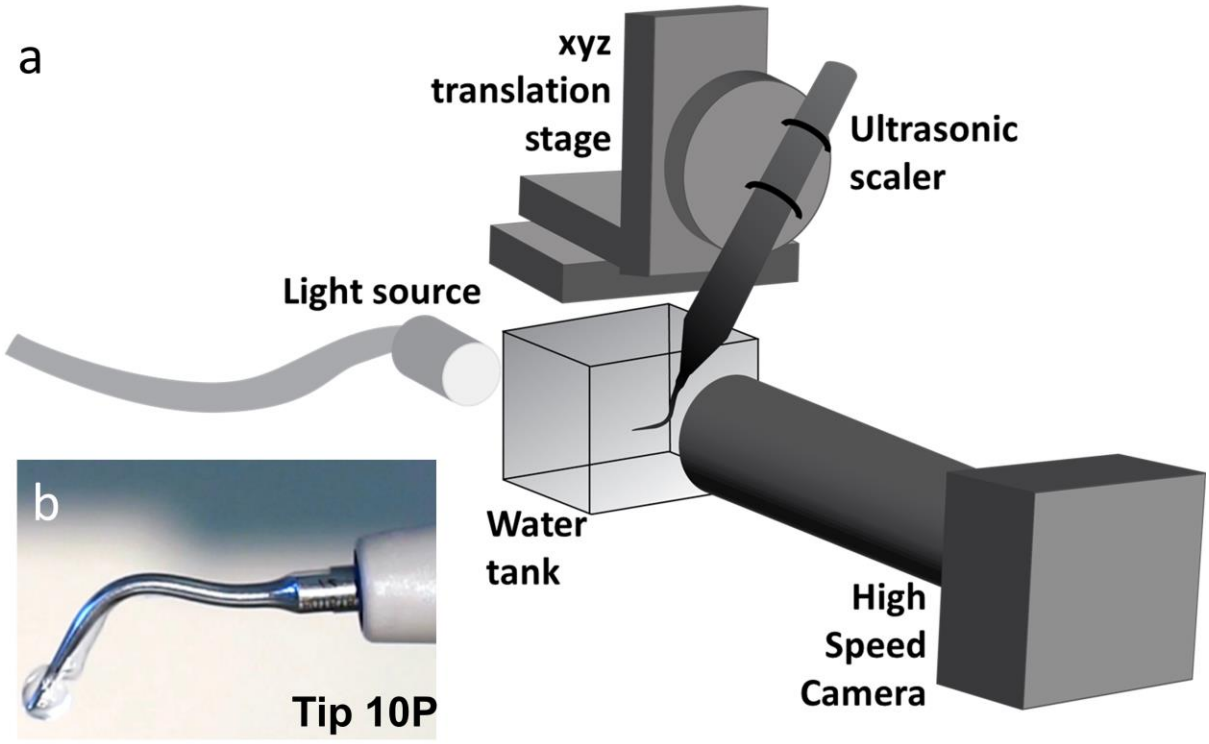

Figure 1. (a) Schematic of experimental setup used for high speed imaging. (b) photograph of the ultrasonic scaler with tip 10P attached, demonstrating how the cooling water flows over the tip during clinical use.

The cavitation generated around the tip was imaged using a high speed camera (Photron Fastcam mini AX200) at 250 and 100,000 frames per second (fps), using a shutter speed of 262 ns. The camera was attached to a long distance microscope zoom lens (12x zoom lens system, Navitar, USA) with a 2x adaptor. For imaging done at 250 fps, a magnification of x1.2 was used, giving a resolution of 8.4 µm/pixel. For imaging done at 100,000 fps, a magnification of x0.58 was used, giving a resolution of 17.2 µm/pixel. The mean time taken for cavitation to start once the scaler had started vibrating was calculated from 5 high speed videos taken at 100,000 fps for each setting.

Image analysis was by Fiji (ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA)[26]. Statistical analysis and data graphing were done using SigmaPlot 14 (Systat Software Inc, San Jose, CA, USA), with statistical significance defined as $p < 0.05$. Testing for statistical significance was done using an ANOVA on ranks test. The Tukey test was then performed to compare all of the settings pairwise.

The mean area of cavitation occurring around the tips at the different carbonation levels was calculated from images taken at 250 fps, from 5 repeats with 300 frames in each repeat. Image analysis was used to calculate the cavitation area (Figure 2). Images were thresholded using the Minimum automatic threshold. The fill holes command was executed to ensure the entire bubble area was segmented. The histogram of each image was then calculated to calculate the number of pixels corresponding to the area of the scaler with the cavitation bubbles. The area of the scaler, which had been calculated with the same method from an image of the scaler before it started vibrating, was then subtracted to leave the area of the cavitation bubbles.

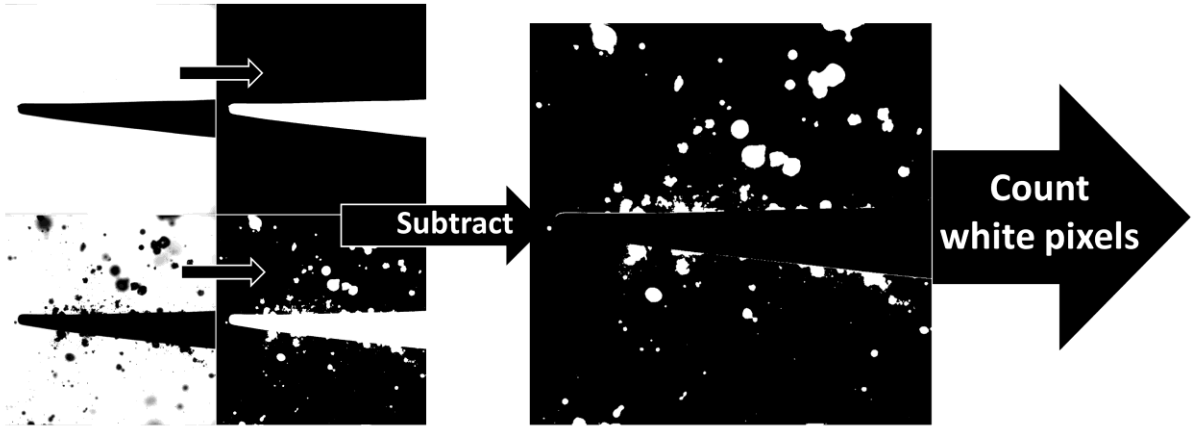

Figure 2 Image analysis steps for calculating the area of cavitation bubbles in high speed videos. Images were binarized using automatic thresholding. The area of the scaler was subtracted from the complete image to calculate the area of the cavitation bubbles. This was done for various settings where the scaler was operated in still water, or water with low, medium or high carbonation, to quantify the amount of cavitation generated at each setting.

Biofilm Growth

The Gram-positive bacteria *Streptococcus sanguinis* (ATCC 10556) was used to form 7 day biofilms. The biofilms were grown on optically transparent polymer coverslips with a surface roughness of 0.02 µm (13 mm, Nunc, ThermoFisher Scientific). This substrate was chosen because of its flexibility compared to glass coverslips, allowing the samples to be positioned vertically inside an imaging tank for high speed imaging, and because of their transparency, to allow for optimal contrast between the background and the biofilm so that image analysis to be conducted more accurately.

The stock microorganisms were recovered from porous storage beads maintained at −80 °C and initially grown on Tryptone Soya Agar (Oxoid, UK) media at 37 ˚C with 5% CO_2 for 3 days. 2-3 single colonies were used to inoculate 10 ml of Brain Heart Infusion (BHI) medium (Oxoid, U.K.) supplemented with 1% sucrose (Fluka Analytical, UK), which was incubated at 37 ˚C, shaking at 88 rpm overnight until it reached approximately 10^9 colony forming units/ml This primary culture was serially diluted to 10^3 cfu/ml in BHI medium.

Artificial saliva was added to the biofilm culture surface to promote biofilm formation, this was prepared according to the method described by Pratten et al.[27] Artificial saliva (1ml) was pipetted into each well of a 24-well plate into which a sterile Thermanox coverslip had been placed and was removed after 15 minutes, to condition the samples. One corner of the coverslips was bent upwards using sterile forceps to create a tab so the samples could be removed from the well with minimal biofilm disruption.

One ml of the diluted *S. sanguinis* culture and 1 ml of fresh BHI medium was added to each well of the 24-well plates. The 24-well plates were then incubated at 37 ˚C, 88 rpm for 24h to allow biofilm formation. The broth was replaced with 2 ml fresh BHI medium every 24 h. The Thermanox coverslips were removed from the 24 well plates after a total of 7 days of incubation and then fixed in 0.1 M sodium cacodylate buffer and 2.5% glutaraldehyde (25% EM grade, Agar Scientific, Essex, UK). They were then stained with Crystal Violet stain (Pro-Lab Diagnostics, UK) for 5 minutes and gently washed in Phosphate Buffered Saline (PBS) (Sigma-Aldrich, USA). Samples were stored in PBS until high speed imaging to prevent dehydration.

A high speed camera (Photron AX200) was used to image the biofilm disruption. The camera was operated at 500 fps with a shutter speed of 1/300,000. The coverslip with biofilm was fixed vertically in a custom-made glass water tank with dimensions 2.7 cm x 2.7 cm x 2.7 cm. The tank was filled with 15 ml reverse osmosis (RO) water or with RO water carbonated at the high setting. The ultrasonic scaler tip was immersed in the glass water tank and its position was fixed 0.5 mm away from the biofilm by attaching it to a XYZ translation stage (PT3, Thorlabs Inc, NJ, USA) and a high-precision rotation mount (PRO1/M, Thorlabs Inc, NJ, USA). The axial rotation of the scaler tip was also maintained during each experiment. The sample was illuminated using an LED cold light source (Hayashi HDF7010, Japan) in bright

field mode. Biofilm removal was imaged while the scaler was operated at the medium power setting for 2s (n = 5).

The total area of biofilm cleaned after using the ultrasonic scaler in still water or in carbonated water was calculated using image analysis. High speed image stills at t = 0 and t = 2 s were thresholded in Fiji using the intermodes automatic threshold. Objects smaller than 20 pixels were removed using the analyse particles plugin to remove noise. The histogram was calculated to obtain the number of pixels corresponding to the cleaned area. The area cleaned was calculated by subtracting the area at t = 0 from the area at t = 2s. This was repeated 5 times using 5 different biofilm samples for each carbonation setting tested.

Results and Discussion

There are 4 main results. Carbonated water caused earlier cavitation inception, more cavitation bubbles around the ultrasonic scaler tip, more bubble cloud lift off and increased biofilm removal.

High Speed Imaging of Cavitation

In carbonated water, the cavitation around the vibrating tip started quicker than in non-carbonated water (Supplementary video a). In still water the tip vibrated for approximately 100 oscillations before cavitation bubbles were seen in the water in high speed videos taken at 100k fps (after approximately 4 ms), whereas when the tips were immersed in carbonated water the cavitation started immediately after the tips started vibrating (after 0.3-0.5 ms, or between 6-15 scaler oscillations).

High speed images showed cavitation around an ultrasonic scaler tip in still water, or in low, medium and high carbonation respectively (

Figure 3 (a-d)). The high speed images and also the area of cavitation calculated using image analysis show that comparing to that in non-carbonated water, significantly more cavitation occurs in carbonated water and the amount of cavitation increases rapidly with the level of carbonation (

Figure 3 (f)). There was a statistically significant difference in the area of cavitation observed among the different test groups (p < 0.001). This is in agreement with previous studies

which have stated that increased gas content facilitates the nucleation of cavitation bubbles [28]. Carbon dioxide has a high solubility in water. Increased gas content reduces the surface tension of the liquid, and the higher the solubility of the gas, the more it can reduce the surface tension, thus facilitating bubble nucleation [22, 28, 29]. Therefore an increased number of cavitation bubbles would lead to more rapid biofilm removal.

The results in the current study agree with studies done around endodontic files, where cavitation occurs in a similar manner to ultrasonic scalers. An increase in cavitation was qualitatively observed around endodontic files when they were operated in carbonated water [30] and in oversaturated water [31], although in these studies quantitative measurements of the cavitation were not made.

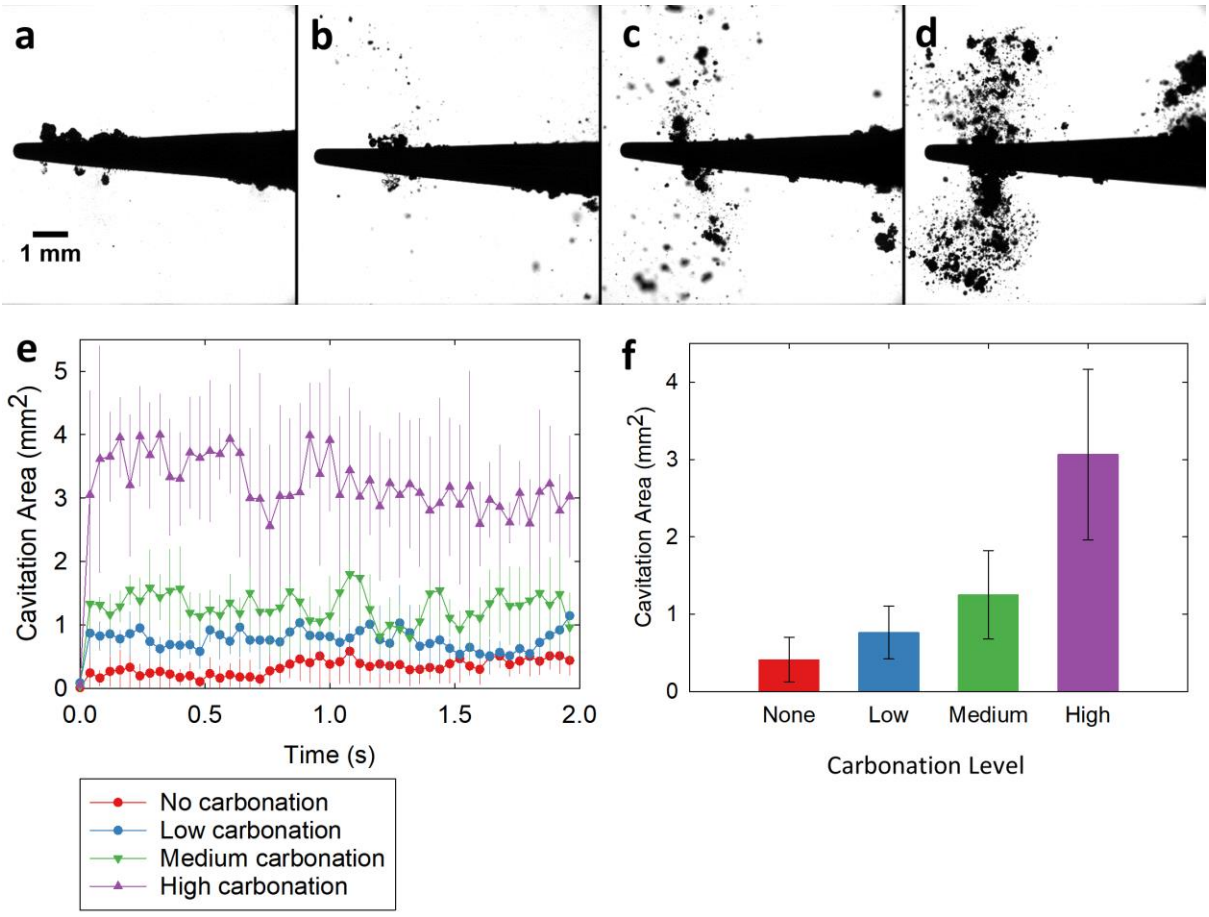

Figure 3 (a-d) High speed video stills showing cavitation around an ultrasonic scaler tip in still water, or in low, medium and high carbonation respectively. (e) Cavitation area around the tip over time at

the different carbonation levels, calculated using image analysis from high speed videos (*n* = 5). (f) Total cavitation area around the tip at the different carbonation levels, calculated using image analysis from the high speed videos.

When the scaler was operated in non-carbonated water, cavitation only occurred at some points along the length of the scaler tip that was visible in the field of view. However, when the scaler was operated in carbonated water, the cavitation occurred along the entire visible length of the scaler tip (supplementary video b). The increase in cavitation observed in the current study demonstrates that cavitation can happen in regions around the scaler where it was not previously possible in non-carbonated water. This could cause increased biofilm removal without the clinician having to move the scaler tip around the implant so less damage is caused.

Inertial cavitation bubbles in the form of cavitation clouds occurred approximately 1 mm radially outward around the scaler tip, and mostly collapsed back onto the tip (Figure 3 and supplementary video b). Non-inertial cavitation bubbles were observed radially 4 mm away from the ultrasonic scaler tip when it was supersaturated with CO_2, where bubbles oscillated repeatedly without collapsing. More bubble lift-off was also observed in carbonated water compared to non-carbonated water, where clusters of bubbles from the cavitation clouds broke away from the cloud and were propelled away from the scaler. Chaotic bubble oscillations and micro-jets were also observed in individual cavitation bubbles.

Biofilm Removal

High speed imaging showed that significantly more biofilm area was removed when the scaler tip was in carbonated water compared to still water after 2s (Figure 4 as well as Supplementary video c). This should be because there was more cavitation happening in carbonated water and this also caused more individual bubbles on the surface of the biofilm which could clean the surface quicker (Figure 5). The orientation of the tip mimics the clinical situation where the body of the probe is held parallel to the biofilm covered surface to allow the tip to vibrate parallel to the surface to prevent damage. The biofilm removal increase seen in the high speed videos was mainly in areas next to the tip, which correlates with the

areas around the tip where the increase in cavitation was observed in high speed imaging. There was a statistically significant difference in the amount of biofilm removed using cavitation in still water compared to when immersed in carbonated water ($p < 0.05$). Surface cleaning through cavitation is thought be due to shear forces exerted on the surface during cavitation bubble collapse. This can occur when the bubble forms a microjet upon collapse or due to acoustic streaming around an oscillating bubble[28]. In the present study the frame rate was not high enough to image microjet formations, but inertial collapsing bubbles were seen on the coverslip and biofilm in high speed imaging (Figure 5), which could be producing microjets upon collapse. There were more inertial collapsing bubbles on the coverslip surface when the tip was operated in carbonated water compared to when in still water, suggesting that the increase in the level of carbonation was able to clean more biofilm.

Previous studies have not investigated carbonated water and cavitation for increased biofilm removal, but recent studies have shown that increased dissolved oxygen in cavitating water does cause increased surface cleaning [20, 22]. Yamashita et al. suggest that cavitation bubbles in oxygen supersaturated water could cause less erosion [22]. This can happen because the gas inside the bubble cushions the implosion of the bubble and so it exerts a milder force on the surface it collapses on. This may also be occurring in carbonated water and may aid in causing less damage to soft tissue such as gum surrounding dental implants, but further work can be done to investigate how the type of gas affects the cleaning ability of cavitation bubbles.

Although there was a significant difference in the biofilm cleaning in still water and carbonated water, the results in the current study were from a sample size of 5 for each condition and there were large differences in the biofilm removal as indicated by the large standard deviation. Therefore, further work can be done to investigate the cleaning efficiency using a larger sample size and on different types of surfaces. The protocols developed in this study can be applied for such studies.

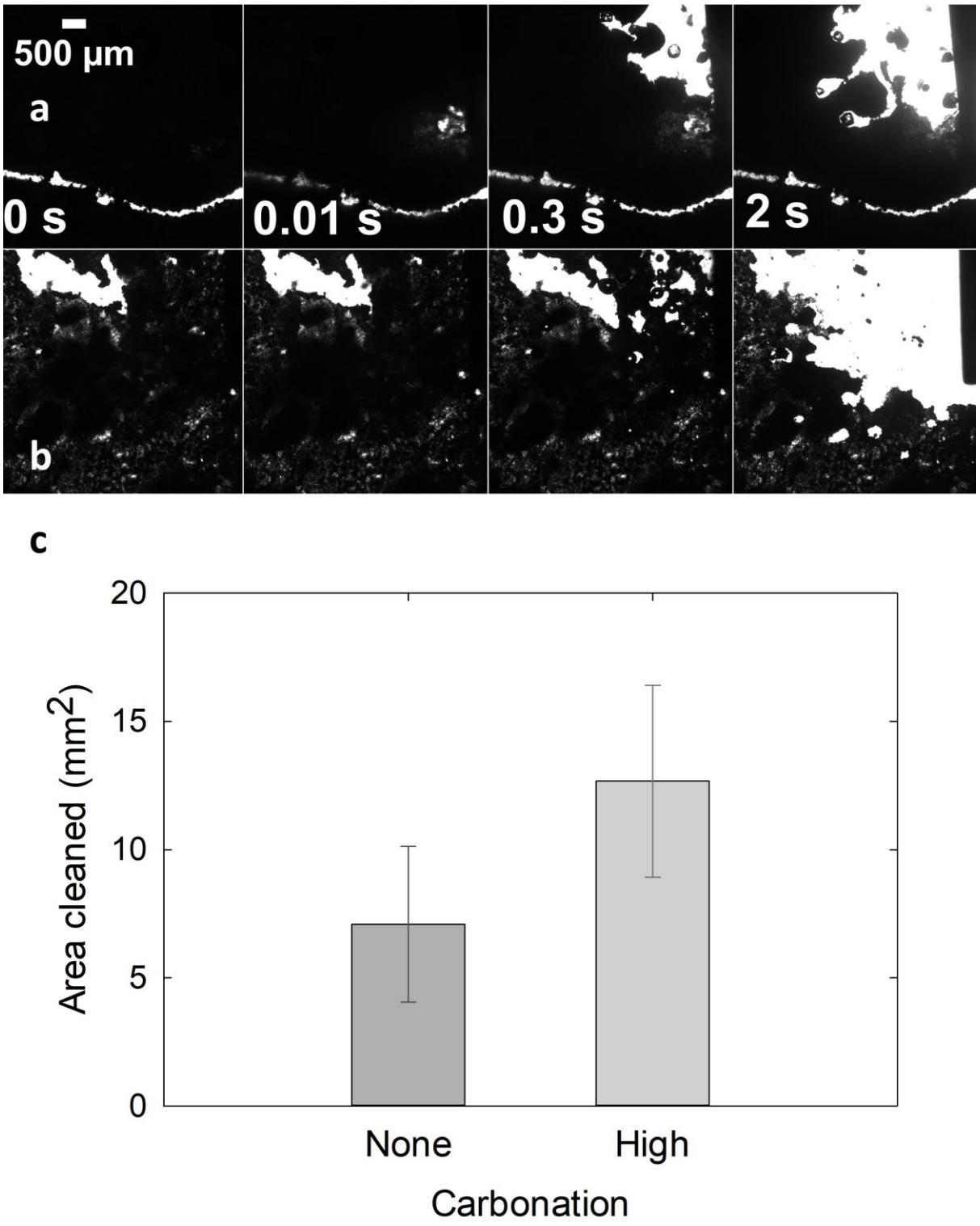

Figure 4 (a) High speed video sequence of biofilm being removed in still water. (b) High speed video sequence of biofilm being removed in carbonated water (c) Biofilm area cleaned when operating the ultrasonic scaler tip in still water or in carbonated water at the high setting, average taken from a series of 5 repeats.

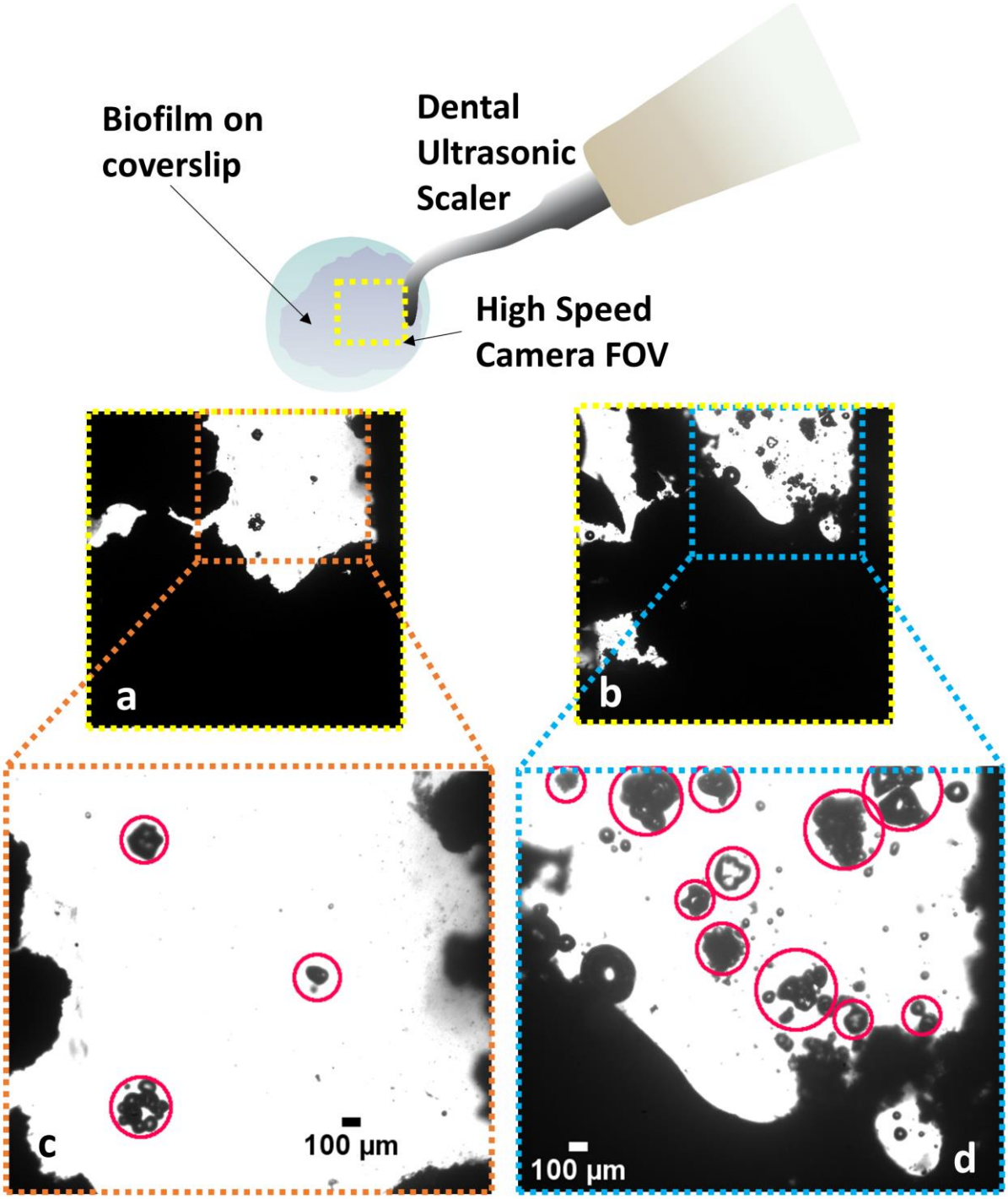

Figure 5 High speed video stills of biofilm being removed. Top: schematic of the experimental setup demonstrating the field of view of the high speed camera. High speed images of biofilm being removed via ultrasonic cavitation in still water (a,c) and in carbonated water (b,d). Cavitating bubbles in chaotic oscillation larger than 100 µm have been circled in red demonstrating that there were more cavitating bubbles at the biofilm surface when the scaler was operated in carbonated water.

The advantages of this study are that the cleaning has been tested on bacterial biofilm using a clinically available instrument, so it is similar to what could occur in a clinical

situation. In addition, carbonated water is safe and non toxic [25], which can also accelerate the translation of this technology. Carbonated water is also very easy to produce so it is a cost effective, easily implementable method. Finally, we have demonstrated two image analysis methods of analysing high speed images which can be easily applied to other similar studies in fluid mechanics, biomedical engineering and water engineering, where the area of cleaning needs to be calculated from a series of high speed images.

One limitation of the study is that the biofilm was grown on a smooth plastic coverslip, which has different attachment properties to rough biomaterial surfaces. The transparent surface was chosen to enable brightfield imaging so there was more intensity contrast between the biofilm and background for more accurate image analysis. Also, in peri-implantitis, the biofilm is typically anaerobic and multi-species, therefore in future work this study can be repeated using a multi species biofilm comprised of bacteria such as *Fusobacterium nucleatum, Porphyromonas gingivalis* and *Actinomyces naeslundii,* on rough titatnium implant-like surfaces.

The dissolved air already present in the water was not purged during the carbonation process, but we do not anticipate this to have major effects on the experiment because the surface tension of water would be effectively reduced by the CO_2, not air. Another limitation is that the scaler clinically would have cooling water running over the tip inside which the cavitation would occur. Due to high speed imaging constraints the tip was immersed inside a water tank in this study, therefore further studies can be carried out with adding carbonated water to the cooling water flow over the tip. If using the scaler in a confined space such as a periodontal pocket, the amount of cavitation nuclei available would be quickly depleted, but if the carbonated water was replenished through the cooling water, this may allow for the increased cavitation to occur continuously, which could remove biofilm more effectively over a long period of time.

The results of this study can be applied for cleaning other objects such as other medical implants. They can also be used in the future for contact-free application of dental ultrasonic scalers for cleaning teeth and dental implants without causing damage.

Conclusions

We have demonstrated that the cavitation occurring around dental ultrasonic scalers increases by operating the scaler tip inside carbonated water and this leads to more biofilm removal via cavitation. Carbonated water caused earlier cavitation inception, more cavitation bubbles around the ultrasonic scaler tip, more bubble cloud lift off and increased biofilm removal. These trends significantly increase with the level of carbonation. This will be useful in cavitation cleaning applications such as for the removal of bacterial biofilms but can also have applications in a wide range of fields where cavitation is used for surface cleaning.

Acknowledgements

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