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Which Parameters affect Biofilm Removal with Acoustic Cavitation? A Review

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Abstract

Bacterial biofilms are a cause of contamination in a wide range of medical and biological areas. Ultrasound is a mechanical energy that can remove these biofilms using cavitation and acoustic streaming, which generates shear forces to disrupt biofilm from its surface. The aim of this narrative review is to investigate the literature on the mechanical removal of biofilm using acoustic cavitation to identify the different operating parameters affecting its removal using this method. The properties of the liquid and the properties of the ultrasound have a large impact on the type of cavitation generated. These include gas content, temperature, surface tension, frequency of ultrasound and acoustic pressure. Many of these parameters require more research to understand their mechanisms in the area of ultrasonic biofilm removal and further research will help to optimise this method for effective removal of biofilms from different surfaces.

Key words: Ultrasonic cleaning; Biofilm Removal; Biofilm Cavitation

Uses of Ultrasonic Biofilm Removal

Biofilm is a coagulated mass of bacterial microorganisms adhered to a surface(2003, Costerton, et al. 1999, Flemming, et al. 2016). Biofilms can form on any non-sterile surface when moisture is present. They are problematic in many areas ranging from oral biofilms in the mouth to biofilm infections on medical devices (Bjarnsholt 2013, Costerton, et al. 1999, Salta, et al. 2016). Therefore, the removal of these biofilms without causing damage to surrounding surfaces such as biomaterials is generating interest (Costerton, et al. 1987, Gupta, et al. 2016, Percival, et al. 2015, Veerachamy, et al. 2014, Wu, et al. 2015). Biofilms are often highly tolerant of traditional antimicrobials such as antibiotics, possibly because it may be difficult for antimicrobials to penetrate into the biofilm structure (Bjarnsholt 2013, Wu, et al. 2015), the antibiotics are only effective on metabolically active bacteria, or the antibiotic action may be antagonised by environmental conditions in the biofilm due to nutrient depletion or the build-up of waste products (Stewart and Costerton 2001). It has been suggested that a combination of antimicrobials with physical biofilm disruption via shear stresses could be an effective biofilm management strategy(Koo, et al. 2017). One method of physically disrupting biofilms is by using ultrasound where it produces phenomena such as cavitation and microstreaming. Cavitation is the generation and collapse of gas or vapour bubbles in a liquid, which can be used to remove debris from surfaces(Young 1999). Cavitation has been investigated for ultrasonic cleaning in a range of industries, for example to remove marine biofouling or food contamination (Chahine, et al. 2016, Fink, et al. 2017, Oulahal, et al. 2007, Salta, et al. 2016). Ultrasonic cavitation can also be used in the healthcare sector to remove biofilms, for example oral biofilms on teeth and dental implants, biofilms on wounds, or biofilms on medical instruments (Birkin, et al. 2015, Chahine, et al.

2016, Chen, et al. 2007, Erriu, et al. 2014, Felver, et al. 2009, Howlin, et al. 2015, Pishchalnikov, et al. 2003, Rivas, et al. 2012, Walmsley, et al. 2010, Walmsley, et al. 2013, Wang and Cheng 2013). Koo et al. highlight the advantages of physical biofilm removal: it reduces the probability of antimicrobial resistance because the physical disruption means that less antimicrobials are required, and physical biofilm removal can be easily combined with various antimicrobial agents or nanoparticles(2017). However, current challenges in this area are that the influence of ultrasound waves on biofilm cleaning is not well understood, and the viscoelasticity of biofilms can make them difficult to disrupt(Koo, et al. 2017, van Wijngaarden 2016, Verhaagen and Rivas 2016). There are a range of cavitation bubble phenomena which are thought to contribute to the cleaning process, such as cloud cavitation, shock waves, micro-jets, microstreamers, acoustic streaming and microstreaming(Verhaagen and Rivas 2016).

Ultrasound has been shown to have both antimicrobial and growth-enhancing effects on bacteria, which have been addressed in other reviews (Erriu, et al. 2014, Yu, et al. 2012). Therefore the specific focus of this review is on the ability of ultrasound to physically disrupt biofilms. The review does not focus on ultrasound contrast agents, but on cavitation due to intrinsic nuclei in the fluid medium. The exact mechanisms of how cavitation can clean surfaces are not fully understood and a consensus is yet to be reached on what parameters are the most important for further investigation, even for simpler cases where a solid surface is being cleaned (Verhaagen and Rivas 2016). The mechanisms of how pressure waves interact with a viscoelastic surface such as biofilm have been studied even less(Koo, et al. 2017). A gap in the knowledge has been identified between the basic cavitation phenomena and the practical applications(Lauterborn and Mettin 2015). Therefore it is important to

understand which parameters will optimise cleaning with cavitation for clinical biofilm removal. The mechanical properties of the ultrasound such as frequency and acoustic pressure, and the properties of the fluid, such as surface tension and temperature, can be tailored for more effective disruption of biofilms. The impact of such parameters on ultrasonic biofilm removal in particular requires more research, and has been addressed in the present review.

Aims and Objectives

The aims of this review are to:

1. Investigate the current literature on mechanical biofilm removal with cavitation and identify what parameters affect ultrasonic biofilm disruption.
2. Determine areas for further research which could increase the amount of cavitation and help to make ultrasonic biofilm removal a more efficient process.

Methods

The Web of Science Core Collection Database was searched using the terms biofilm cavitation ultraso*, medical cavitation ultraso*, dental ultraso*, bubble* biofilm, biofilm removal cavitation, ultrasonic cleaning, cavitation clean and ultraso* biofilm from 1980 to July 2018. The factors considered when searching the literature involved the inclusion of English language articles which studied the use of ultrasonic cavitation to mechanically remove bacterial biofilms from surfaces. Accordingly, studies which used ultrasound probes without cavitation for biofilm removal have not been included. Studies where cavitation was not generated acoustically were also excluded. In

addition, ultrasound studies which evaluated the antibacterial effect of ultrasound on bacteria and biofilm have not be included as the focus of the review is on the ability of cavitation and associated bubble dynamics to mechanically disrupt biofilms.

Acoustic cavitation Cleaning Bubble Dynamics

Acoustic cavitation occurs when the local pressure of a liquid falls below the saturated vapour pressure (SVP), which can happen when ultrasound is applied to the fluid(Young 1999). This negative pressure required to form a cavity in a liquid is called the cavitation threshold (Lauterborn and Mettin 2015). When this occurs, during the rarefaction phase of the propagating ultrasound wave, bubbles grow from small pockets of gas (nuclei) present in the liquid (Brennen 2013). The bubbles grow until the ultrasound wave reaches the compression phase, when the pressure increases (Plesset and Prosperetti 1977). This forces bubble oscillation. There are two types of cavitation: inertial or non-inertial (Plesset and Prosperetti 1977) (Figure 1).

In non-inertial cavitation, bubbles oscillate repeatedly at low energy (Lauterborn and Mettin 2015). This pulsation usually occurs when the bubbles are in a low amplitude sound field (Leighton 2012).

If the cavitation is inertial, bubbles repeatedly collapse with high energy and regrow during each cycle, in which the radius of the bubble expands to at least twice the initial size. Due to this limited period, there is no mass transport of permanent gas through the bubble-liquid interface. This lack of gas causes transient bubbles to implode very violently, releasing high amplitude shock waves and high velocity micro jets upon collapse (Brennen 2013, Leighton 2012). They can also fragment into smaller bubbles upon collapse(Leighton 2012). If the ultrasound is closer to the resonance frequency of a non-inertial

157 cavity, it can turn into an inertial cavity over time, when it stops oscillating
158 around an equilibrium radius and starts to grow via rectified diffusion (Young
159 1999). Inertial cavitation is more likely to occur at a higher acoustic pressure
160 amplitude (Izadifar, et al. 2018), although other factors also determine
161 whether cavitation will be inertial or non-inertial, such as host fluid properties,
162 initial bubble size and acoustic frequency.
163 A range of bubble and fluid flow phenomena which may be contributing to
164 biofilm disruption have been identified (Figure 2), although it is unknown
165 exactly which of these are occurring during biofilm removal. The current
166 mechanisms identified as contributing to cavitation cleaning are described
167 below.
168 When a cavitation bubble is generated near a rigid boundary, a liquid jet is
169 formed during bubble collapse that penetrates the opposite bubble wall and
170 impacts the boundary (Figure 2). Physically the jet is caused by a high pressure
171 zone at the base of the jet which occurs during the collapse of the bubble. It is
172 believed that the jet imposes a localised high shear force on the surrounding
173 biofilm, lifting it off the surface (Verhaagen and Rivas 2016). The combined
174 effect of many microbubbles can break up and detach particles from a surface.
175 Blake et al. showed that a bubble must be attached to the surface for the jet to
176 strike the boundary (Blake and Gibson 1987). Ohl et al. speculated that the
177 microjet has the largest influence on bubble cleaning because it causes a
178 higher shear stress at the surface (Ohl, et al. 2006). However, they used laser
179 generated bubbles which may behave differently to acoustically generated
180 cavitation bubbles.
181 Cavitation clouds are clusters of cavitating bubbles (Figure 2). High velocity
182 microjets can only help to clean when emitted by the bubbles on the edge of
183 the cavitation cloud if it is in contact with the biofilm, as microjets within the

184 cloud are directed towards the surrounding bubbles. van Wijngaarden et al.
185 concluded that cleaning from cavitation bubble clouds mainly occurs from the
186 generation of shock waves from the collapsing bubbles (2016), however
187 Lauterborn et al. point out that bubble dynamics in cavitation clouds and
188 clusters needs further research(2015).
189 Shock waves are another major contributor to surface cleaning and are
190 generated during the collapse of an inertial bubble. They can fragment the
191 biofilm on the surface to remove it, but they also have the potential to
192 damage the surface being cleaned, so to prevent this their intensity should be
193 controlled (Verhaagen and Rivas 2016).
194 Microstreamers are ribbons of cavitating microbubbles. They are affected by
195 Bjerknes forces and can migrate towards a pressure node or a pressure
196 antinode, depending on their size in relation to the bubble resonant radius
197 (Leighton, et al. 1990, Wu, et al. 2013). Reuter et al. found using high speed
198 imaging that streamers impacted the surface to be cleaned perpendicularly
199 and may aid in the cleaning (2017). They also showed that bubbles which were
200 in contact with the surface contributed to cleaning.
201 Acoustic streaming is fluid flow caused by momentum transfer from the
202 acoustic wave to the liquid it is propagating in (Nowak, et al. 2015) with a
203 range up to the order of cm(Boluriaan and Morris 2003, Wiklund, et al. 2012).
204 It may assist in the removal of biofilm which is loosely attached to a surface
205 due to the generation of drag forces and shear forces. Microstreaming is fluid
206 flow occurring around growing and collapsing cavitation bubbles, at a similar
207 range to that of the cavitation bubble diameter (Brotchie, et al. 2009,
208 Lamminen, et al. 2004, Leighton 1995). If the biofilm is within this range, it can
209 be dislodged by drag forces produced by the microstreaming flow(Lamminen,
210 et al. 2004). In addition, microstreaming and acoustic streaming transport

211 detached debris away from the surface that is being cleaned(Lamminen, et al.
212 2004).
213 Biofilm removal using cavitation is affected by properties of the fluid, the
214 ultrasound and the biofilm. For example, increasing the viscosity of the fluid
215 increases the cohesive forces between the molecules. Therefore this raises the
216 cavitation threshold as the pressure required for a bubble to grow has to
217 overcome these forces (Chemat, et al. 2017). The following sections outline
218 how the properties of the fluid and the ultrasound affect the amount of
219 cavitation occurring, and show how altering these properties will influence
220 biofilm removal.

221 **Methods of Quantifying Biofilm Removal**

222 To evaluate the efficiency of a method which physically disrupts biofilms, it is
223 important to accurately calculate efficiency of biofilm disruption. This has been
224 done with biological methods such as measuring the dried biomass, semi-
225 quantitative staining, protein/DNA quantifying, or using standard microbial
226 culture techniques to assess the remaining viable bacteria(Hadi, et al. 2010,
227 John, et al. 2014, Kite, et al. 2004, Park, et al. 2013, Qian, et al. 1996). Biofilm
228 removal efficiency has also been determined directly using imaging techniques
229 such as confocal laser scanning microscopy, light microscopy, bioluminescence
230 imaging, scanning electron microscopy and macroscale photography(Agarwal,
231 et al. 2014, Clegg, et al. 2006, Cruz, et al. 2011, Fricke, et al. 2012, Hägi, et al.
232 2015, Li, et al. 2012, Nance, et al. 2013, Salles, et al. 2007, Schwarz, et al. 2006,
233 Sedgley, et al. 2004, Tawakoli, et al. 2015, Vickery, et al. 2004, Whittaker, et al.
234 1984, Wu, et al. 2011, Zhang and Hu 2013). Many studies use imaging
235 techniques qualitatively, or semi-quantitatively, for example by segmenting the
236 images using manual thresholding, which leads to high operator bias(Cruz, et

al. 2011, Salles, et al. 2007, Schwarz, et al. 2006). However recent studies have used more accurate segmentation methods such as machine learning, which are reproducible and not prone to operator-induced variability(Vyas, et al. 2016).

Fluid Properties Contributing to Ultrasonic Biofilm Removal

Surface Tension

Adding a surfactant reduces the surface tension of the liquid and lowers the cavitation threshold because the cohesive forces between the molecules of the liquid are weaker. Therefore the pressure drop has to be lower for cavitation nuclei to grow during the rarefaction stage(Chemat, et al. 2017). As cavitation is happening at a lower pressure amplitude, less power is applied(Chemat, et al. 2017).

Single cavitation bubbles grow at a faster rate when a surfactant is added to water (Ashokkumar and Grieser 2007). Multiple cavitation bubbles grow via two methods: rectified diffusion and bubble coalescence. Adding a surfactant reduces the number of coalescence events, so bubbles mainly grow via rectified diffusion. Consequently more time is required for the same amount of active cavitation bubbles to build up. Yet there is still a larger number of cavitation bubbles when a surfactant is present compared to water (Ashokkumar and Grieser 2007). Further research can be done to investigate different surfactants and how they affect biofilm removal for such purposes.

Gas Content

Cavitation bubbles can grow from gases inside the liquid which behave as nuclei(Brennen 2013). The effect of adding microbubbles to the liquid whilst

applying ultrasound to increase cavitation and hence promote biofilm removal has been the subject of investigation.

The presence of microbubbles or dissolved gas lowers the cavitation threshold and allows cavitation to occur quicker and at a lower power(Caupin and Herbert 2006, Cracknell 1980, Halford, et al. 2012). In addition, applying ultrasound will cause a liquid to degas(Chemat, et al. 2017). When a free cavitation bubble grows, gases dissolve into it because of the low pressure gradient(Chemat, et al. 2017). When the bubble collapses, its surface area decreases so rapidly that the gas inside does not have time to escape and dissolve back into the liquid(Chemat, et al. 2017). Therefore by adding gas bubbles the amount of nucleation sites will not be depleted when ultrasound is applied.

To increase the stability of bubbles in water, encapsulated microbubbles can be used instead of free air bubbles (Wiklund, et al. 2012). They have a gas core encased in a stabilising shell composed of a protein, lipid, polymer or surfactant. Encapsulated microbubbles have traditionally been used as contrast agents in ultrasound imaging and have also been researched for drug delivery (Kiessling, et al. 2012, Liu, et al. 2006). Research has also been conducted on the use of encapsulated microbubbles combined with antibiotics for enhanced antimicrobial efficacy on biofilms (Dong, et al. 2018, Dong, et al. 2017, Halford, et al. 2012, He, et al. 2011, Zhu, et al. 2013). Goh et al. experimentally showed that biofilm could be disrupted using ultrasound combined with microbubble contrast agents (Goh, et al. 2014).

Halford et al. used a high speed camera to image bubbles formed in an artificial dental root canal when ultrasound was applied(2012). Biofilm is difficult to remove from root canals due to their irregular shape, therefore cavitation bubbles may be able to disrupt bacteria from such surfaces more

effectively. Halford et al. observed larger bubbles when the root canal models were inside a microbubble emulsion compared to water, although they do not specify the exact diameters of the bubbles observed. The microbubble emulsion also contained the surfactant Triton X-100, therefore it is unclear whether the increased bubble size occurred due to the gas content or the surface tension and further work using microbubbles with different outer shell compositions can be done to understand this. In addition, the surfactant Triton X-100 is untypical to produce microbubbles and it is toxic to tissue (Jahan, et al. 2008, Koley and Bard 2010). Halford et al. also repeated the experiment with *Enterococcus faecaclis* biofilm, which they removed from root canals using the microbubble emulsion in combination with ultrasonic agitation from an endodontic file. They found less colony forming units compared to the control (no treatment), which indicates either more biofilm being mechanically removed due to the cavitation or an antibacterial effect of the microbubble emulsion.

Dong et al. compared biomass after treating biofilms with 1 MHz ultrasound only or with ultrasound combined with encapsulated microbubbles(2017). The acoustic intensity was 0.5 W/cm^2 and the duty cycle was 50%. Biomass was measured by drying, staining with Crystal Violet and measuring the absorbance of the samples after treatment. They found that there was less biofilm remaining compared to the untreated controls when microbubbles were used with the ultrasound and suggested that the microbubbles could have reduced the cavitation threshold. Crystal Violet staining is a standard test to determine the amount of biofilm(Christensen, et al. 1985), however not washing and heating the biofilms to 65°C before staining in their test is not typical and this may have adhered previously unattached (planktonic) bacteria into the biofilm, altering the results. In addition, samples in their test were not measured

before treatment because crystal violet staining is not compatible with that, so there is the assumption that the control biofilms grew the same as the treated biofilms, although in this paper the controls showed minimal variability. Agarwal et al. have done a similar study using 5-10µm diameter microbubbles inside an ultrasonic bath operating at 42 kHz (2014). They measured the fixed biomass of samples by calculating the dried mass of the biofilm. Using this method it is not possible to compare the same samples before and after treatment and the difference in the amount of initial biofilm on each sample could have altered the results. However, as above, Agarwal et al. did show the variability in the untreated control biofilms to be minimal. They found that there was 75% less biomass compared to the untreated controls when ultrasound was used in combination with microbubbles, whilst only 10% less biomass with only ultrasound and 38% less biomass with only microbubbles. Agarwal et al. also noted that the microbubbles disappeared 2s after applying the ultrasound pulse(2014).

Microbubbles were applied continuously for 15 minutes, while ultrasound was applied for 2 seconds every 2 minutes during the microbubble sparging

The advantages of this method are that the cavitation is less likely to cause damage because it is applied intermittently. The disadvantage is that it would be difficult to apply this method clinically, where rapid biofilm removal within a few seconds is desired.

Liu et al. suggest that a high bubble density as well as gas filled bubbles can hinder cavitation by causing acoustic attenuation which results in energy loss(2014). In addition, because the bubbles are filled with gas rather than vapour, their collapse strength is lower because the gas cushions the implosion (Capote and de Castro 2007, Hammitt 1980, Liu, et al. 2014). Birkin et al. noticed in experiments that the sound speed changes in the range of 868-1063

342 ms^{-1} associated with a strongly cavitating field as the void fraction of gas
343 around 2.9 x 10^{-3} to 4.2 x 10^{-3} % (2003). However further study is needed to
344 find the threshold in terms of the volume fraction occupied by bubbles in the
345 liquid. As some gas bubbles are required to act as nucleation points for
346 cavitation inception, Liu et al. suggest that the optimal oxygen content range
347 for cavitation is 3.17 to 5.12 mgL^{-1} (2014). Ferrell et al. noted that it is more
348 likely to have microjets and shock waves in partially degassed water (2002).
349 Many of the studies evaluated have not specified the gas content of the fluid
350 used, therefore it is unclear how much of an effect this has on increasing
351 biofilm removal. It would be useful to conduct studies where the gas content is
352 varied from a degassed state through to adding microbubbles to the fluid to
353 determine which concentration results in more biofilm removal. Robinson et
354 al. studied this using an artificial biofilm model and found no difference in the
355 amount of cavitation occurring between tap water and water with added
356 microbubbles, but found that less cavitation occurred in degassed
357 water(2017). This is as expected, since the cavitation threshold would have
358 increased. There was no significant difference in the amount of artificial biofilm
359 removed when using the degassed water in comparison to the saturated
360 water. This was measured by using image analysis to calculate the area of
361 hydrogel in each frame of a high speed video.
362 The removal will also depend on the biofilm and its attachment to the
363 substrate, since a larger force would be required to detach biofilm which has a
364 higher adhesive strength. Therefore further work could be done on biofilms
365 with different levels of attachment and to find the optimum gas content of the
366 fluid to maximise its removal with cavitation. This approach will allow
367 ultrasonic cavitation to be optimised for specific biofilm removal applications.
368 For clinical applications such as dental cleaning or superficial wound

debridement, a device can be used to add gas bubbles to the water before it is delivered to the area to be cleaned.

The type of gas inside the bubble also affects the cavitation collapse force and therefore the cleaning ability. For encapsulated microbubbles, a fluorocarbon gas core is typically used because it has a low diffusion coefficient to enable stability against dissolution (Wiklund, et al. 2012). For free bubbles, a more soluble gas will lower the surface tension more and cause more bubble nucleation(Rooze, et al. 2013). However this may cause less intense bubble collapse, so experiments with different gas mixtures can be conducted to understand how they influence biofilm removal (Rooze, et al. 2013).

Vapour Pressure and Temperature

The vapour pressure, defined as the pressure of a vapour in contact with its liquid form, can affect the force of the bubble implosion. The collapse of a cavitation bubble is less intense in high vapour pressure solvents, due to the stronger cushioning effect of the vapour with high vapour pressure (Chivate and Pandit 1995). The selection of the liquid medium depends on the type of application (Gogate and Pandit 2001). Applications such as biofilm removal from tissue *in vivo* need less intense cavitation to prevent tissue damage so liquids with a higher vapour pressure can be used. Liquids with a lower vapour pressure can be used for applications such as biofilm removal from rigid biomaterials or surgical instruments, which can withstand more intense cavitation. The vapour pressure can be lowered by decreasing the temperature of the fluid, but this also causes its surface tension and viscosity to increase, which raises the cavitation threshold (Chemat, et al. 2017).

Cavitation occurs most intensely between 7 to 20 ˚C and radically decreases above 30 ˚C (Niemczewski 2014). This is thought to be because water degasses

when heated so there are less cavitation nuclei present(Niemczewski 2014). As acoustic energy in a liquid can be dissipated into heat energy, cavitation will cause the liquid to heat up. Therefore in biofilm removal experiments, the temperature must be monitored to ensure that the cavitation is not affected (Capelo-Martínez 2009).

What remains unclear is to what degree the temperature affects ultrasonic biofilm elimination. Therefore further work can be done to measure the amount of biofilm disruption at different temperatures, using the temperature range given by Niemczewski et al. as a guideline (2014). Although it may be difficult to control temperature for some clinical applications, such as for *in vivo* biofilm removal, it may be feasible in others, for example where an ultrasonic bath is used to remove biofilm from surgical instruments.

Ultrasound Properties Contributing to Ultrasonic Biofilm Removal

Type of Ultrasound: Transducer/Probe

Different mechanisms have been researched to deliver acoustic ultrasound to biofilms. Some of these include a high intensity focussed ultrasound (HIFU) beam (Bigelow, et al. 2009, Khoo, et al. 2016, Xu, et al. 2012), an ultrasound transducer immersed in the fluid(Lombardo, et al. 2017, Mott, et al. 1998, Nishikawa, et al. 2010, Oulahal, et al. 2007, Thiruppathi, et al. 2014, Xu, et al. 2012, Zips, et al. 1990), a sonotrode/ultrasonic probe: a rod vibrated ultrasonically at its resonant frequency (Cracknell 1980, Gartenmann, et al. 2017, Macedo, et al. 2014, Vyas, et al. 2016) and an acoustically activated water stream(Birkin, et al. 2015, Howlin, et al. 2015, Salta, et al. 2016).

The advantage of using ultrasound transducers combined with a water tank or an ultrasound bath for biofilm removal is that the experimental setup is easier, and the frequency and power can be easily altered by using different

transducers. This could translate clinically to remove biofilm from surgical instruments and medical devices. However this method would not be feasible for use in all applications, for example in removing biofilm from medical devices such as prosthetic joints and dental implants *in vivo,* because it would be difficult to immerse these in liquid(Birkin, et al. 2015).

Cavitation can also be delivered through a narrow ultrasonic probe/horn immersed in a solution (Capelo-Martínez 2009). The vibrating probe then generates cavitation inside the fluid it is immersed in. Examples include dental applications such as ultrasonic scalers and endodontic files. In these applications the cavitation is not currently used clinically but it is being researched as a biofilm removal method (Ahmad, et al. 1988, Gartenmann, et al. 2017, Macedo, et al. 2014, Pecheva, et al. 2016, Thurnheer, et al. 2014, Van der Sluis, et al. 2007, Vyas, et al. 2016, Walmsley 1988) (Figure 3). The disadvantage of using a narrow probe/horn is that the cavitation intensity rapidly decreases with distance, since its disturbance to the liquid flow decays rapidly away from the probe/horn. Nevertheless this could prevent collateral damage.

The Starstream instrument (Ultrawave, Cardiff, UK) uses an ultrasonically activated water stream to remove biofilm (Birkin, et al. 2015, Howlin, et al. 2015, Salta, et al. 2016). The main advantage of this is that it does not restrict the size of the object, enabling biofilm removal from larger objects which cannot fit inside an ultrasonic bath(Salta, et al. 2016). Biofilms and proteins can shield microorganisms on medical devices from sterilisation (Hadi, et al. 2010). The Starstream device can remove protein on surgical stainless steel surfaces as well as oral biofilms *in vitro* (Birkin, et al. 2015). It is likely that the Starstream could also be used for biofilm removal from surgical instruments, although further research is needed to confirm this. A disadvantage of this

technique is that it is not suitable for small applications where the location of the cavitation must be precisely controlled, such as for dental debridement. It also uses large volumes of water (2L/min), therefore for use in the mouth a lower water flow rate is required.

Blondin et al. compared an ultrasonic bath to an ultrasonic probe for extracting biofilms from sand and found that the probe was more effective for this application (2001). Similar research could be done in other areas by evaluating the removal effectiveness using a transducer and a probe to determine which method is most effective.

Frequency and Intensity

A higher cavitation intensity (>10 W/cm^2) is required to remove biofilms attached to a surface (Erriu, et al. 2014). Low frequency ultrasound (<500 kHz as defined by Erriu et al. (2014)) produces more intense cavitation because there is more time for bubbles to grow during the rarefaction phase of the ultrasound (Chemat, et al. 2017, Izadifar, et al. 2018).

In the kHz range, different ultrasound frequencies have been investigated to determine which causes the most effective cleaning. Mott et al. investigated the effect of frequency on the amount of biofilm removed. Using infra-red absorbance measurements before and after application of the ultrasound, they found that ultrasound at 20 kHz (~7 W transducer power, two 30s pulses) removed more biofilm than ultrasound at 33 kHz and 150 kHz (35-40 W transducer power) (1998). Further studies investigating frequency changes between 10 to 200 kHz could help to determine whether there are differences in biofilm removal at this lower frequency range, as this range is used during ultrasonic cleaning of surfaces (Fuchs 2015).

Acoustic Pressure

A study has investigated the effect of cavitation at 26 kHz with different acoustic pressure amplitudes in the range 36-76 kPa (Kim and Kim 2014). High speed imaging showed that the pressure amplitude affected the bubble structures. Micro-jets were only seen when the acoustic pressure was lower than the non-inertial pressure value. When the acoustic pressure increased, it either caused oscillation of spherical bubbles (non-inertial cavitation), or inertial cavitation , where bubble clouds were observed. High speed imaging has shown that inertial cavitation occurs around dental ultrasonic scaler tips and endodontic files and this is able to disrupt biofilm(Van der Sluis, et al. 2007, Vyas, et al. 2016). The effect of changing the acoustic pressure on biofilm removal has not yet been investigated therefore further research in this area could help to improve cleaning efficiency, for example by conducting high speed imaging studies of biofilm removal to visualise the effect of the different bubble structures on the disruption of the biofilm structures. This would give further information on how inertial and non-inertial cavitation bubbles affect biofilms and which types of bubbles cause the most biofilm removal.

Biofilm Properties Contributing to Ultrasonic Biofilm Removal

Viscoelasticity

As most biofilms are viscoelastic, the bubble dynamics may be different compared to near a rigid boundary. The biofilm properties will also have an effect on its removal. Viscoelastic biofilms can deform when forces are applied without detaching from the surface (Macedo, et al. 2014), therefore larger shear forces may be required for removal. Studies have measured the biofilm

adhesive pressures, for example using microbead force spectroscopy(Lau, et al. 2009), but these have not been compared to the acoustic pressure and the pressures generated by the different cavitation cleaning mechanisms. Research is required in this area to give insights into the specific mechanisms in action during biofilm removal as well as to aid in identifying the optimal ultrasound parameters for elimination of different biofilms.

Some fundamental cavitation research has shown that a bubble developing a jet near a single thin elastic membrane points away from the boundary, as would happen near a free surface. Near a stiff membrane (high elasticity), bubble jets point towards the membrane as would happen near a rigid boundary. Therefore mineralised biofilms such as dental calculus may behave as a rigid boundary. (Brujan, et al. 2001)(Shervani-Tabar, et al. 2013). Ohl et al. modelled the dynamics of a non-equilibrium bubble near hard and soft tissues using a simplified model (Ohl, et al. 2009). They found that the Young's modulus, Poisson ratio and density affected the bubble dynamics. When near soft tissue the bubble spilt into smaller bubbles, which formed opposing jets under certain conditions. Near hard tissue, the bubbles formed jets which collapsed towards the surface. Curtiss et al. investigated the interaction between a bubble and a tissue layer (Curtiss, et al. 2013). They describe how a toroidal bubble can re-expand, causing tissue to peel away from a rigid surface. Further research can be done on this to understand the interactions between microjets and biofilms. In addition, the gap between the applications of cavitation cleaning and fundamental cavitation research can be closed further by researching into acoustic cavitation with fluid flow(Lauterborn and Mettin 2015).

Limitations of the review

A limitation inherent to the design of this review is that studies on the antibacterial effect of ultrasound on biofilms were excluded, along with studies on the enhancement of antibiotic effects on biofilms with ultrasound. These may also have observed a mechanical removal effect that has not been included in this review. In addition, only studies using acoustically generated cavitation have been included. Other methods such as laser generated cavitation and shockwaves have not been included in this review on acoustic cavitation but they may also disrupt biofilm.

Areas Where Further Research is Needed

This narrative review showed that there is much debate on the mechanisms underlying the ability of ultrasonic cavitation to clean surfaces. Further research should be directed in this area to assist in the understanding of the disruptive effect on biofilms. Specifically, further research on biofilm removal with acoustic cavitation can be done using liquids with lower surface tension or different gas contents, or at low temperatures or low acoustic frequencies, as these factors can increase cavitation. It is also important to observe and understand how fluid flow and associated stresses affect biofilm removal. More realistic physical and numerical models are expected for simulating the intricate interactions among the dynamics of cavitation bubbles, the associated liquid flows and the deformation and removal of biofilm. Advanced imaging techniques and correlative imaging can be effectively used to investigate these, although if imaging techniques are used quantitatively, it is important to use image-processing methods that ensure accurate quantification of biofilm removal.

Conclusion

Cavitation is an unpredictable phenomenon but when it occurs it has a strong disruptive action on the biofilm and the research on this topic will lead to enhanced biofilm removal techniques in healthcare applications. It is important to optimise cavitation by influencing the different parameters such as bubble collapse intensity and activity within the fluid that the ultrasound is generated in and this requires further research in understanding the mechanisms involved.

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Figure Legends

Figure 1: Schematic showing the processes of inertial cavitation (a) and non-intertial cavitation (b) taking place when ultrasound is applied. Reproduced with permission from Izadifar et al. (2018)

Figure 2: Schematic showing the different methods in which cavitation can cause mechanical biofilm removal. The white arrow indicates the direction of ultrasound insonification. Micro-jets point away from the biofilm if it is soft (low elasticity), as shown in the figure, but they point towards the biofilm if it is 'stiff' (high elasticity) (e.g. mineralised biofilms).

Figure 3: Scanning electron microscopy images of *S. mutans* biofilm on dental implant-type surfaces, before and after treatment with an ultrasonic scaler at low power (no cavitation occurring) (**a**,**b**) and medium power (cavitation occurring)(**c**,**d**). The blue overlay shows the automatic detection of bacteria. Reproduced from Vyas et al. (2016)

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