

Which parameters affect biofilm removal with acoustic cavitation?

Vyas, Nina; Manmi, K.; Wang, Qian; Jadhav, A. J.; Sammons, Rachel; Barigou, M.; Sammons, R. L.; Kuehne, Sarah; Walmsley, Anthony

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1 **Which Parameters affect Biofilm Removal with Acoustic Cavitation? A Review**

2 N. Vyas¹, K. Manmi², Q. X. Wang², A. J. Jadhav³, M. Barigou³, R. L. Sammons¹, S.
3 A. Kuehne¹, A.D. Walmsley^{1*}

4 1. School of Dentistry, College of Medical and Dental Sciences, University of
5 Birmingham, Mill Pool Way, Birmingham, B5 7EG, U.K.

6 2. School of Mathematics, College of Engineering and Physical Sciences,
7 University of Birmingham, B15 2TT, U.K.

8 3. School of Chemical Engineering, College of Engineering and Physical
9 Sciences, University of Birmingham, B15 2TT, U.K.

10 * Corresponding Author

11 Corresponding author details:

12 Telephone +44 (0) 121 466 5493 (Secretary); Fax +44 (0) 121 237 2825

13 Email a.d.walmsley@bham.ac.uk

14 The School of Dentistry, College of Medical and Dental Sciences

15 University of Birmingham

16 5 Mill Pool Way

17 Edgbaston, Birmingham, UK

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28 **Abstract**

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

42 Key words: Ultrasonic cleaning; Biofilm Removal; Biofilm Cavitation

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52 **Uses of Ultrasonic Biofilm Removal**

53 Biofilm is a coagulated mass of bacterial microorganisms adhered to a
54 surface(2003, Costerton, et al. 1999, Flemming, et al. 2016). Biofilms can form
55 on any non-sterile surface when moisture is present. They are problematic in
56 many areas ranging from oral biofilms in the mouth to biofilm infections on
57 medical devices (Bjarnsholt 2013, Costerton, et al. 1999, Salta, et al. 2016).
58 Therefore, the removal of these biofilms without causing damage to
59 surrounding surfaces such as biomaterials is generating interest (Costerton, et
60 al. 1987, Gupta, et al. 2016, Percival, et al. 2015, Veerachamy, et al. 2014, Wu,
61 et al. 2015). Biofilms are often highly tolerant of traditional antimicrobials
62 such as antibiotics, possibly because it may be difficult for antimicrobials to
63 penetrate into the biofilm structure (Bjarnsholt 2013, Wu, et al. 2015), the
64 antibiotics are only effective on metabolically active bacteria, or the antibiotic
65 action may be antagonised by environmental conditions in the biofilm due to
66 nutrient depletion or the build-up of waste products (Stewart and Costerton
67 2001). It has been suggested that a combination of antimicrobials with physical
68 biofilm disruption via shear stresses could be an effective biofilm management
69 strategy(Koo, et al. 2017). One method of physically disrupting biofilms is by
70 using ultrasound where it produces phenomena such as cavitation and
71 microstreaming. Cavitation is the generation and collapse of gas or vapour
72 bubbles in a liquid, which can be used to remove debris from surfaces(Young
73 1999). Cavitation has been investigated for ultrasonic cleaning in a range of
74 industries, for example to remove marine biofouling or food contamination
75 (Chahine, et al. 2016, Fink, et al. 2017, Oulahal, et al. 2007, Salta, et al. 2016).
76 Ultrasonic cavitation can also be used in the healthcare sector to remove
77 biofilms, for example oral biofilms on teeth and dental implants, biofilms on
78 wounds, or biofilms on medical instruments (Birkin, et al. 2015, Chahine, et al.

79 2016, Chen, et al. 2007, Erriu, et al. 2014, Felver, et al. 2009, Howlin, et al.
80 2015, Pishchalnikov, et al. 2003, Rivas, et al. 2012, Walmsley, et al. 2010,
81 Walmsley, et al. 2013, Wang and Cheng 2013). Koo et al. highlight the
82 advantages of physical biofilm removal: it reduces the probability of
83 antimicrobial resistance because the physical disruption means that less
84 antimicrobials are required, and physical biofilm removal can be easily
85 combined with various antimicrobial agents or nanoparticles(2017). However,
86 current challenges in this area are that the influence of ultrasound waves on
87 biofilm cleaning is not well understood, and the viscoelasticity of biofilms can
88 make them difficult to disrupt(Koo, et al. 2017, van Wijngaarden 2016,
89 Verhaagen and Rivas 2016). There are a range of cavitation bubble phenomena
90 which are thought to contribute to the cleaning process, such as cloud
91 cavitation, shock waves, micro-jets, microstreamers, acoustic streaming and
92 microstreaming(Verhaagen and Rivas 2016).

93 Ultrasound has been shown to have both antimicrobial and growth-enhancing
94 effects on bacteria, which have been addressed in other reviews (Erriu, et al.
95 2014, Yu, et al. 2012). Therefore the specific focus of this review is on the
96 ability of ultrasound to physically disrupt biofilms. The review does not focus
97 on ultrasound contrast agents, but on cavitation due to intrinsic nuclei in the
98 fluid medium. The exact mechanisms of how cavitation can clean surfaces are
99 not fully understood and a consensus is yet to be reached on what parameters
100 are the most important for further investigation, even for simpler cases where
101 a solid surface is being cleaned (Verhaagen and Rivas 2016). The mechanisms
102 of how pressure waves interact with a viscoelastic surface such as biofilm have
103 been studied even less(Koo, et al. 2017). A gap in the knowledge has been
104 identified between the basic cavitation phenomena and the practical
105 applications(Lauterborn and Mettin 2015). Therefore it is important to

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

113 **Aims and Objectives**

114 The aims of this review are to:

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

121 **Methods**

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

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

135 **Acoustic cavitation Cleaning Bubble Dynamics**

136 Acoustic cavitation occurs when the local pressure of a liquid falls below the
137 saturated vapour pressure (SVP), which can happen when ultrasound is applied
138 to the fluid(Young 1999). This negative pressure required to form a cavity in a
139 liquid is called the cavitation threshold (Lauterborn and Mettin 2015). When
140 this occurs, during the rarefaction phase of the propagating ultrasound wave,
141 bubbles grow from small pockets of gas (nuclei) present in the liquid (Brennen
142 2013). The bubbles grow until the ultrasound wave reaches the compression
143 phase, when the pressure increases (Plesset and Prosperetti 1977). This forces
144 bubble oscillation. There are two types of cavitation: inertial or non-inertial
145 (Plesset and Prosperetti 1977) (Figure 1).

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

149 If the cavitation is inertial, bubbles repeatedly collapse with high energy and
150 regrow during each cycle, in which the radius of the bubble expands to at least
151 twice the initial size. Due to this limited period, there is no mass transport of
152 permanent gas through the bubble-liquid interface. This lack of gas causes
153 transient bubbles to implode very violently, releasing high amplitude shock
154 waves and high velocity micro jets upon collapse (Brennen 2013, Leighton
155 2012). They can also fragment into smaller bubbles upon collapse(Leighton
156 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

237 al. 2011, Salles, et al. 2007, Schwarz, et al. 2006). However recent studies have
238 used more accurate segmentation methods such as machine learning, which
239 are reproducible and not prone to operator-induced variability(Vyas, et al.
240 2016).

241 **Fluid Properties Contributing to Ultrasonic Biofilm Removal**

242 Surface Tension

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

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

258 Gas Content

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

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

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

273 To increase the stability of bubbles in water, encapsulated microbubbles can
274 be used instead of free air bubbles (Wiklund, et al. 2012). They have a gas core
275 encased in a stabilising shell composed of a protein, lipid, polymer or
276 surfactant. Encapsulated microbubbles have traditionally been used as
277 contrast agents in ultrasound imaging and have also been researched for drug
278 delivery (Kiessling, et al. 2012, Liu, et al. 2006). Research has also been
279 conducted on the use of encapsulated microbubbles combined with antibiotics
280 for enhanced antimicrobial efficacy on biofilms (Dong, et al. 2018, Dong, et al.
281 2017, Halford, et al. 2012, He, et al. 2011, Zhu, et al. 2013). Goh et al.
282 experimentally showed that biofilm could be disrupted using ultrasound
283 combined with microbubble contrast agents (Goh, et al. 2014).

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

288 effectively. Halford et al. observed larger bubbles when the root canal models
289 were inside a microbubble emulsion compared to water, although they do not
290 specify the exact diameters of the bubbles observed. The microbubble
291 emulsion also contained the surfactant Triton X-100, therefore it is unclear
292 whether the increased bubble size occurred due to the gas content or the
293 surface tension and further work using microbubbles with different outer shell
294 compositions can be done to understand this. In addition, the surfactant Triton
295 X-100 is untypical to produce microbubbles and it is toxic to tissue (Jahan, et al.
296 2008, Koley and Bard 2010). Halford et al. also repeated the experiment with
297 *Enterococcus faecalis* biofilm, which they removed from root canals using the
298 microbubble emulsion in combination with ultrasonic agitation from an
299 endodontic file. They found less colony forming units compared to the control
300 (no treatment), which indicates either more biofilm being mechanically
301 removed due to the cavitation or an antibacterial effect of the microbubble
302 emulsion.

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

315 before treatment because crystal violet staining is not compatible with that, so
316 there is the assumption that the control biofilms grew the same as the treated
317 biofilms, although in this paper the controls showed minimal variability.

318 Agarwal et al. have done a similar study using 5-10 μ m diameter microbubbles
319 inside an ultrasonic bath operating at 42 kHz (2014). They measured the fixed
320 biomass of samples by calculating the dried mass of the biofilm. Using this
321 method it is not possible to compare the same samples before and after
322 treatment and the difference in the amount of initial biofilm on each sample
323 could have altered the results. However, as above, Agarwal et al. did show the
324 variability in the untreated control biofilms to be minimal. They found that
325 there was 75% less biomass compared to the untreated controls when
326 ultrasound was used in combination with microbubbles, whilst only 10% less
327 biomass with only ultrasound and 38% less biomass with only microbubbles.
328 Agarwal et al. also noted that the microbubbles disappeared 2s after applying
329 the ultrasound pulse(2014).

330 Microbubbles were applied continuously for 15 minutes, while ultrasound was
331 applied for 2 seconds every 2 minutes during the microbubble sparging
332 The advantages of this method are that the cavitation is less likely to cause
333 damage because it is applied intermittently. The disadvantage is that it would
334 be difficult to apply this method clinically, where rapid biofilm removal within a
335 few seconds is desired.

336 Liu et al. suggest that a high bubble density as well as gas filled bubbles can
337 hinder cavitation by causing acoustic attenuation which results in energy
338 loss(2014). In addition, because the bubbles are filled with gas rather than
339 vapour, their collapse strength is lower because the gas cushions the implosion
340 (Capote and de Castro 2007, Hammitt 1980, Liu, et al. 2014). Birkin et al.
341 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 2.9×10^{-3} to 4.2×10^{-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

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

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

379 Vapour Pressure and Temperature

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

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

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

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

407 **Ultrasound Properties Contributing to Ultrasonic Biofilm Removal**

408 Type of Ultrasound: Transducer/Probe

409 Different mechanisms have been researched to deliver acoustic ultrasound to
410 biofilms. Some of these include a high intensity focussed ultrasound (HIFU)
411 beam (Bigelow, et al. 2009, Khoo, et al. 2016, Xu, et al. 2012), an ultrasound
412 transducer immersed in the fluid(Lombardo, et al. 2017, Mott, et al. 1998,
413 Nishikawa, et al. 2010, Oulahal, et al. 2007, Thiruppathi, et al. 2014, Xu, et al.
414 2012, Zips, et al. 1990), a sonotrode/ultrasonic probe: a rod vibrated
415 ultrasonically at its resonant frequency (Cracknell 1980, Gartenmann, et al.
416 2017, Macedo, et al. 2014, Vyas, et al. 2016) and an acoustically activated
417 water stream(Birkin, et al. 2015, Howlin, et al. 2015, Salta, et al. 2016).

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

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

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

438 The Starstream instrument (Ultrawave, Cardiff, UK) uses an ultrasonically
439 activated water stream to remove biofilm (Birkin, et al. 2015, Howlin, et al.
440 2015, Salta, et al. 2016). The main advantage of this is that it does not restrict
441 the size of the object, enabling biofilm removal from larger objects which
442 cannot fit inside an ultrasonic bath (Salta, et al. 2016). Biofilms and proteins can
443 shield microorganisms on medical devices from sterilisation (Hadi, et al. 2010).
444 The Starstream device can remove protein on surgical stainless steel surfaces
445 as well as oral biofilms *in vitro* (Birkin, et al. 2015). It is likely that the
446 Starstream could also be used for biofilm removal from surgical instruments,
447 although further research is needed to confirm this. A disadvantage of this

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

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

457 Frequency and Intensity

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

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

473 Acoustic Pressure

474 A study has investigated the effect of cavitation at 26 kHz with different
475 acoustic pressure amplitudes in the range 36-76 kPa (Kim and Kim 2014). High
476 speed imaging showed that the pressure amplitude affected the bubble
477 structures. Micro-jets were only seen when the acoustic pressure was lower
478 than the non-inertial pressure value. When the acoustic pressure increased, it
479 either caused oscillation of spherical bubbles (non-inertial cavitation), or
480 inertial cavitation , where bubble clouds were observed. High speed imaging
481 has shown that inertial cavitation occurs around dental ultrasonic scaler tips
482 and endodontic files and this is able to disrupt biofilm(Van der Sluis, et al.
483 2007, Vyas, et al. 2016). The effect of changing the acoustic pressure on
484 biofilm removal has not yet been investigated therefore further research in
485 this area could help to improve cleaning efficiency, for example by conducting
486 high speed imaging studies of biofilm removal to visualise the effect of the
487 different bubble structures on the disruption of the biofilm structures. This
488 would give further information on how inertial and non-inertial cavitation
489 bubbles affect biofilms and which types of bubbles cause the most biofilm
490 removal.

491

492 **Biofilm Properties Contributing to Ultrasonic Biofilm Removal**

493 Viscoelasticity

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

499 adhesive pressures, for example using microbead force spectroscopy(Lau, et al.
500 2009), but these have not been compared to the acoustic pressure and the
501 pressures generated by the different cavitation cleaning mechanisms. Research
502 is required in this area to give insights into the specific mechanisms in action
503 during biofilm removal as well as to aid in identifying the optimal ultrasound
504 parameters for elimination of different biofilms.

505 Some fundamental cavitation research has shown that a bubble developing a
506 jet near a single thin elastic membrane points away from the boundary, as
507 would happen near a free surface. Near a stiff membrane (high elasticity),
508 bubble jets point towards the membrane as would happen near a rigid
509 boundary. Therefore mineralised biofilms such as dental calculus may behave
510 as a rigid boundary. (Brujan, et al. 2001)(Shervani-Tabar, et al. 2013). Ohl et
511 al. modelled the dynamics of a non-equilibrium bubble near hard and soft
512 tissues using a simplified model (Ohl, et al. 2009). They found that the Young's
513 modulus, Poisson ratio and density affected the bubble dynamics. When near
514 soft tissue the bubble spilt into smaller bubbles, which formed opposing jets
515 under certain conditions. Near hard tissue, the bubbles formed jets which
516 collapsed towards the surface. Curtiss et al. investigated the interaction
517 between a bubble and a tissue layer (Curtiss, et al. 2013). They describe how a
518 toroidal bubble can re-expand, causing tissue to peel away from a rigid surface.
519 Further research can be done on this to understand the interactions between
520 microjets and biofilms. In addition, the gap between the applications of
521 cavitation cleaning and fundamental cavitation research can be closed further
522 by researching into acoustic cavitation with fluid flow(Lauterborn and Mettin
523 2015).

524 **Limitations of the review**

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

533 **Areas Where Further Research is Needed**

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

549

550 **Conclusion**

551

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

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563

564 Figure Legends

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

568

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

574

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

580

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