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Biomedical Applications for Gas-Stabilizing Solid Cavitation Agents

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1 **Abstract**

2 For over a decade, advancements in ultrasound-enhanced drug delivery strategies have
3 demonstrated remarkable success in providing targeted drug delivery for a broad range of diseases.
4 In order to achieve enhanced drug delivery, these strategies harness the mechanical effects from
5 bubble oscillations (i.e., cavitation) of a variety of exogenous cavitation agents. Recently, solid
6 cavitation agents have emerged due to their capacity for drug-loading and sustained cavitation
7 duration. Unlike other cavitation agents, solid cavitation agents stabilize gaseous bubbles on
8 hydrophobic surface cavities. Thus, the design of these particles are crucial. In this review, we
9 provide an overview of the different designs for solid cavitation agents such as nanocups,
10 nanocones, and porous structures as well as the current status of their development. Considering
11 the numerous advantages of solid cavitation agents, we anticipate further innovations for this new
12 type of cavitation agent across a broad range of biomedical applications.

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6 22 **Introduction**
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9 23 Ultrasound is a well-established imaging modality and is rapidly becoming an attractive
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11 24 therapeutic modality for ablation¹ , histotripsy² , and enhanced drug delivery³ owing to its
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13 25 controllability, safety, and cost effectiveness. Though therapeutic ultrasound utilizes the thermal
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15 26 effects of ultrasound (e.g., drug release from liposomes⁴), there has been a substantial interest in
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17 27 taking advantage of the mechanical effects from cavitation. Effects such as microstreaming⁵,
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19 28 jetting⁶, and shockwave formation⁷, play a key role in the disruption of both cells and tissue in
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22 29 order to enable enhanced uptake of a therapeutic or its complete destruction. For example,
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24 30 ultrasound has been shown to temporarily open the blood brain barrier by applying shear stress
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26 31 onto the endothelial cells and allowing for therapeutics to bypass the tight junctions⁸. Similarly,
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28 32 ultrasound has been shown to induce sufficient mechanical force to completely disrupt and lyse
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30 33 blood clots⁹.
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34 34 Cavitation provides the mechanical effects necessary to induce the bioeffects attributed to the
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36 35 broad-range of ultrasound-enhanced therapies. Yet, to nucleate cavitation requires substantial
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38 36 acoustic energy. At these acoustic intensities, cavitation nucleates stochastically, causing
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40 37 indiscriminant damage to both healthy and diseased tissue. For applications such as noninvasive
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42 38 tumour histotripsy, the lethal intensities of ultrasound are desired. However, these acoustic
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44 39 intensities are not wanted for ultrasound-enhanced drug delivery strategies in order to minimize
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46 40 off-target drug transport. Thus, exogenous gaseous bodies are introduced as cavitation nuclei to
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48 41 lower the requisite acoustic power for cavitation. These cavitation agents come in many forms, but
49
50 42 generally may be categorized as microbubbles, phase-change nanodroplets, echogenic liposomes,
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52 43 and more recently solid cavitation nuclei.
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Conventional cavitation agents such as microbubbles have shown great potential for drug-loading and localized drug delivery. Yet, these cavitation agents are often limited by their size and inability to extravasate beyond the vasculature¹⁰. Furthermore, microbubbles are often destroyed by ultrasound, though there are efforts in devising improved ultrasound conditions to reduce bubble destruction¹¹. Thus to overcome these limitations, researchers have developed solid cavitation agents that are capable of stabilizing bubbles within cavities on the particles. Considering the growing interest in solid cavitation agents, we aim to provide an overview on the current trends in the design and application of solid cavitation agents in this report.

Cavitation

Cavitation refers to an acoustic phenomena whereby the local pressure variances from ultrasound induce an oscillatory motion of a bubble or group of bubbles¹²⁻¹³. This motion may be broadly characterized as either governed by the acoustic pressure oscillations (i.e., non-inertial or stable cavitation), or by the inertia of the surrounding fluid (i.e., inertial cavitation)¹⁴. Both types of cavitation have been shown to enhance drug delivery, though there are some functional differences in application¹⁵. Stable cavitation occurs when bubbles oscillate over several acoustic cycles about their equilibrium radius. Thus, lower acoustic intensities are typically used, and these oscillations are greatly influenced by the driving frequency of the ultrasound transducer¹⁶⁻¹⁷. The radial oscillations from stable cavitation bubbles have been applied to enhance micro-mixing¹⁸ and drug delivery to tissue and cells¹⁹. It has also shown a remarkable capability to reversibly permeate membranes such as the blood-brain barrier to facilitate drug transport²⁰. Though the local streaming may further disrupt cell aggregation proximal to the cavitation bubble¹⁷, stable cavitation has been shown to be safe and bioeffects being temporary²¹.

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3 66 In contrast, inertial cavitation refers to a bubbles unconstrained expansion and subsequent inertial
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5 67 collapse. The violent collapse of the bubbles results in shockwave generation²², high velocity fluid
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7 68 jets¹³, free radical formation²³, and intense microstreaming²⁴. Furthermore, sustained inertial
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9 69 cavitation enhances heating and mechanical disruption in tissue. As a result, inertial cavitation has
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11 70 been used to improve drug delivery deep into tissue and enhance chemical transport of small to
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13 71 large drug molecules into cells via sonoporation¹⁵.
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18 72 **Nucleation of Cavitation**
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21 73 Cavitation plays a key role for several non-thermal biomedical applications. Nowadays,
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23 74 controlling the inception of cavitation in tissue is challenging owing to its stochastic nature.
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25 75 Uncontrolled cavitation has been shown to produce unintended side effects in mammalian
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27 76 tissues²⁵. Thus, exogenous cavitation nuclei or cavitation agents are often injected to reduce the
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29 77 acoustic power necessary to induce cavitation, thereby spatially and temporally control
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31 78 cavitation²⁶. Historically, these cavitation agents are typically micrometric surfactant stabilized
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33 79 gaseous particles, i.e., microbubbles, coated with a variety of materials (e.g., lipids, polymers, and
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35 80 proteins). Microbubbles have seen much success as ultrasound contrast agents (Albunex ®,
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37 81 Definity ®, Optison TM, etc.) and are recognized as promising candidates for numerous therapeutic
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39 82 applications²⁷. However, microbubbles are relatively large (1 to 10 µm in diameter), which may
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41 83 be considered too big for entering target tissue through vasculature in disease such as cancer¹⁰. As
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43 84 a result, researchers have sought out to generate sub-micron cavitation agents.
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49 85 To reach sub-micrometer sizes, another cavitation nuclei design has been employed. A droplet
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51 86 comprised of a perfluorocarbon with a liquid to vapor phase-transition temperature close to body
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53 87 temperature transform into bubbles after exposure to a particular amplitude and frequency of
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55 88 ultrasound²⁸⁻²⁹. Because these particles are initially liquid, their sizes may reach as small as 100's
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of nanometers, and have thus been shown to enter into the microvasculature of tumours³⁰. Furthermore, perfluorocarbon nanodroplets have also been shown to increase the delivery of vancomycin to methicillin resistant *Staphylococcus aureus* biofilms³¹. Yet despite these advantages, phase transitioning nanodroplets are limited by the larger acoustic power necessary to vaporize the droplets. The acoustic power needed for vaporization increases as the size of nanodroplet decreases³².

Both microbubbles and phase-change nanodroplets have demonstrated marked success in various therapeutic applications. These technologies remain restricted to the vasculature and are ultimately destroyed by ultrasound. As a result, solid cavitation agents have come forward to provide sustained cavitation and enable extravasation beyond the blood vessels. Furthermore, there are also numerous of in-depth review articles on the topic of microbubbles and phase-change nanodroplets^{29, 33} that the authors encourage the readers to look through as they will not be further discussed in this review. Instead, this review will turn its focus on the emerging technology of solid cavitation agents.

Mechanism and Design of Solid Cavitation Agents

There are several factors to be considered for designing solid cavitation agents. Unlike fluid-based cavitation agents that contain the bubble within the core of the particle, solid cavitation agents typically stabilize or nucleate gas within pores or crevices on the surface or throughout the particle. This concept was first postulated by Harvey et al.³⁴ in 1944 and further explored by others³⁵, where it was suggested that a hydrophobic crevice may allow for a gas nuclei that tend to dilate rather than collapse due to surface tension. Another theory proposes that in the presence of a sudden drop in pressure (e.g., the expansion phase of ultrasound), gas bubbles are formed from the detachment of the liquid phase from the surface of the solid (i.e., tribonucleation)³⁶. Considering these

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mechanisms for bubble formation, there are two critical design criteria for solid cavitation agents,
1) the shape of the particles and 2) the hydrophobicity of the material.

Mesoporous Silica

The demand for porous materials such as mesoporous silica began in the early 1990's due to their improved catalytic activity. Since, mesoporous silica nanoparticles (MSN) with different pore sizes have been formulated for drug delivery and other applications³⁷. Recently, the development of solid cavitation agents such as mesoporous silica nanoparticles (MSN)³⁸⁻³⁹ and mineralized nanoparticles⁴⁰⁻⁴¹ have been shown to be capable of gas stabilization and bubble nucleation. For porous particles, the negative pressure from ultrasound increases the volume of the gas, hence pull the bubble out of the port. Since the pores neighbor each other, the gas pockets coalesce to form larger bubbles capable of both stable and inertial cavitation.

One of the earlier developments of MSN as cavitation agents was conducted by Wang and colleagues⁴². They produced a hydrophobic MSN (HMSN) by mixing tetraethyl orthosilicate (TEOS) with cetyltrimethylammonium bromide (CTAB), a pore forming agent. Briefly, CTAB was mixed with NaOH and deionised water. After stirring at 80°C, TEOS was added dropwise. CTAB was removed by washing with ethanol. MSN obtained was rendered hydrophobic by adding hexamethyldisilazane (HMDS) in n-hexane. The HMSN had a hexagonal structure with 5 nm diameter internal hydrophobic nanovoids, and had a mean diameter of 220 nm in water. The nanovoids were filled by air, as the water was unable to enter the pores due to lower interfacial free energy that favored the gas phase. Beta cyclodextrin was also added to coat the HMSN with a hydrophilic surface and facilitate stability in water. Cavitation was observed during exposure to low energy ultrasound (LEUS) at an intensity below 1 W cm⁻² and a frequency range between 1 and 3 MHz. Combined LEUS and HMSN treatment of a ZR75-30 breast cancer cell line group

135 showed up to 10% cell viability at 50 ug/ml concentration after only 20 seconds of ultrasound
 136 irradiation. Furthermore, it was evident that the survival rates of the cells depended on intensity,
 137 irradiation time, and concentration of HMSN. Histopathology revealed that the cell death was due
 138 to coagulative necrosis primarily from the effects of cavitation; pristine HMSN showed more than
 139 70% cell viability. This was also observed in a xenograft tumour mouse model, whereby LEUS
 140 treatment with HMSN reduced tumour sizes by up to 55% with visible cell necrosis. Interestingly,
 141 these studies were conducted without the need to a chemotherapeutic, further indicating the ability
 142 for cavitation to produce an anti-tumour effect.

143 Following this work, a variation of HMSN was created with perfluorocarbon loaded nanovoids
 144 that nucleate bubble for ultrasound imaging. Instead of beta-cyclodextrin, pluronic F127 was used
 145 as capping agent⁴³. Pluronic HMSN (P-hMSN) were synthesized by tetraethyl orthosilicate co-
 146 condensation with octyltriethoxysilane and cetyltrimethylammonium bromide, producing particles
 147 with a 100 nm diameter and 3 nm diameter pores (Figure 1). As observed in HMSN, P-hMSN
 148 entrap air inside the void due the hydrophobicity of the silica. Unlike HMSN, P-hMSN were
 149 produced as contrast agents for blood pool imaging. After P-hMSN were added to fetal bovine
 150 serum and whole blood, cavitation was still observable, though there was found to be a drop in
 151 signal of 20% and 35% respectively compared to that of signal in PBS⁴⁴. The reason for signal
 152 reduction in serum and blood compared to PBS was stated to be due to higher density and
 153 scattering in blood.

Figure 1. (A) Air entrapment in hydrophobic pores of P-hMSN. (B) Ultrasound mediated bubble generation in P-hMSN. Reprinted with permission from⁴⁴. DOI: 10.1002/adhm.201600030 and copyright 2016 Wiley.

Interestingly, it has been shown that the hydrophobicity of the surface contributes to the formation of cavitation. For example, MSNs produced with relatively low hydrophobicity (as measured by water drop contact angle) required acoustic intensities at 7.5 MHz frequencies at approximately 3.3 MPa peak negative pressure to induce cavitation. In contrast, super-hydrophobic MSNs displayed cavitation at 1.6 MPa peak negative pressures at the same frequency. Furthermore, cavitation from super-hydrophobic MSNs were more intense and remained active for longer periods of time compared to MSNs. These results suggested that the hydrophobicity of the MSNs played a critical role regarding the nucleation of cavitation and its subsequent intensity and duration⁴⁵.

HMSN are able to store large amounts of drugs due to the large number of pores, and have thus been loaded with therapeutic agents to enhance targeted delivery. Considering the previous work on HMSN, the cavitation thresholds of FITC labelled HMSN were investigated using single element focused ultrasound transducers with center frequencies of 0.5 and 1.6 MHz. At 0.5 MHz broadband signal detection from a passive cavitation detection device indicated that inertial cavitation was first observed at 0.6 MPa, but did not become consistently detected until 2 MPa. As expected, the inertial cavitation thresholds required more intense pressure amplitudes at higher frequencies (1.6 MHz) with consistent cavitation nucleation occurring at 4.3 MPa peak negative pressure amplitude. In an agarose flow through phantom, FITC conjugated HMSN were shown to extravasate beyond the flow channel after exposure to ultrasound (Figure 2). Interestingly, there was more directional penetration of the particles at 1.6 MHz compared to 0.5 MHz⁴⁶.

Figure 2. Mesoporous silica nanoparticles (MSN) implantation in agarose flow chamber mold at 1.6 MHz and 4 MPa ultrasound parameters. Reprinted with permission from⁴⁶. DOI: 10.1016/j.cej.2017.12.051 and copyright 2018 Elsevier.

Silica based janus nanoparticles have also been synthesized. These janus porous silicon (J-PSi) nanoparticles have hydrophobic inner pores with a hydrophilic outer surface. The hydrophobic pores of J-PSi trap the gas necessary for the nucleation of cavitation at reduced cavitation threshold acoustic pressure amplitudes. In order to improve biocompatibility and reduce particle aggregation⁴⁷, the outer surface of the J-Psi was PEGylated, resulting in particles with diameters of around 150 nm and pore size of 12 nm. Under continuous ultrasound irradiation J-PSi showed contrast enhancement even at 5 ug/ml concentration⁴⁸. Using a computational model for bubble oscillations in an acoustic field, it was estimated that the bubbles formed in response to ultrasound were approximately the size of conventional contrast agents. However, compared to the clinically available contrast agent (SonoVue), the contrast enhancement from J-Psi was lower. This was attributed to the fact that bubbles must be pulled from the pores of the particles before being detectable under pulsed ultrasound. Therefore the short cycles used for diagnostic B-mode ultrasound do not provide sufficient time for bubble nucleation. As a result, it may be that using longer cycles first to nucleate the bubbles will provide better contrast enhancement in B-mode ultrasound (Figure 3).

Figure 3. (A) Janus PSi NP fabrication and PEGylation process for ultrasound contrast enhancement. (B) Average intensity plot from B-mode image. Oxidised porous silicone nanoparticle is denoted as O-PSi and Pegylated oxidised porous silicone nanoparticles as PEG-O-PSi. Reprinted with permission from⁴⁸. DOI: 10.1021/acsami.7b11007 and copyright 2017 American Chemical Society.

A number of pharmaceuticals have poor water solubility, thus limiting their feasibility if directly injected into the patient. Considering that HMSNs may be tuned for a variety of surface topographies, pore sizes, and surface chemistry, these structures have also been favored as drug carriers to enhance the bioavailability of hydrophobic drugs⁴⁹⁻⁵². Presently, a common technique for drug loading into HMSNs is a solvent evaporation method, whereby the particles and drug are first mixed in an organic solvent. As the solvent evaporates, a concentration gradient of the drug between the MSNs and external solution is created, thereby enhancing drug uptake by the MSNs. He et al. assessed the drug uptake using ethanol dichloromethane (DCM) and dimethylsulfoxide (DMSO). The group demonstrated that solvent evaporation using DCM allowed for the highest uptake of the drug as the solvent polarity was much less than the drug, minimizing competition for surface adsorption with the MSNs. These particles demonstrated enhanced cancer cell cytotoxicity relative to the free drug despite a drug loading efficiency of 21% on average⁴⁹. This method not only relies on concentration gradients for drug loading, but also the hydrophobicity of the drug. For example, Stewart et al. recently presented a method for co-assembly of MSNs with a hydrophilic drug within the structure. The group demonstrated increased drug loading with their approach as well as longer sustained release of their drug, ultimately enhancing the therapeutic effect of their drug and system⁵³. While the group utilized a hydrophilic drug, it has been shown that some pharmaceuticals may be modified to achieve similar effects, potentially improving the

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3 222 prospect of enhanced drug delivery for a number of therapeutic agents⁵⁴. Ultrasound augmented
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5 223 release of drug from MSN have also been established. By grafting an ultrasound responsive
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7 224 copolymer with an ultrasound labile acetal structure on MSN surface, doxorubicin (DOX)
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9 225 controlled release was achieved. In this study, the copolymer acted as a gatekeeper that closed and
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11 226 opened the pores based on physiological temperature, preventing unspecific release. Ultrasound
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13 227 irradiation also modulated the hydrophobicity of the copolymer and enabled site specific release
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15 228 of drug. The ultrasound effect on pore opening was analyzed at 0.5, 1 and 3.3 MHz frequencies
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17 229 and was found that inertial cavitation from ultrasound was responsible for this effect⁵⁵. Few other
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19 230 works with MSN loaded with drug also directly linked acoustic cavitation to be the main factor for
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21 231 drug release⁵⁶. Even though the studies related to ultrasound responsive particles for drug release
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23 232 were relatively less compared to other external stimuli responsive particles⁵⁶⁻⁵⁷, the results from
24
25 233 existing research shows that the notion of drug-loaded solid cavitation agents is definitely a
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27 234 promising area to explore.
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36 236 **Polymer Nanocups**

37 237 Polymer-based particles have also been considered as solid cavitation nuclei. Unlike the silica
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39 238 particles, which trap gas within the porous structure, polymer particles have a cup-like shape, and
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41 239 are often referred to in the literature as nanocups. To produce these particles, a seed
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43 240 polymersisation method was employed, whereby polystyrene nanoparticles were coated with
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45 241 methyl methacrylate and 1,2-divinylbenzene to induce swelling and deformation of the
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47 242 nanoparticle (Figure 4A). As a result, size-adjustable sub-micron particles were produced with
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49 243 uniform size distribution and a single well-defined surface cavity.
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Figure 4. Polymeric nanocup as solid cavitation agent. (A) Nanocup synthesis and mechanism of cavitation nucleation (B) HepG2 tumor reduction by systemic co-injection of oncolytic virus and nanocup with intratumor injection of prodrug. Reprinted with permission from⁵⁸⁻⁵⁹. DOI:10.1002/sml.201501322 and copyright 2015 Wiley. DOI:10.1038/mt.2016.139 and copyright 2016 CellPress. Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

The presence of a surface cavity on the nanocup allowed for bubbles to be trapped onto the surface of the particle. A modified Rayleigh-plesset crevice model was used to predict the response of the surface cavity trapped bubble in response to ultrasound⁶⁰. The model suggested that these bubbles expanded out of the particle, detached, and subsequently underwent inertial collapse. Interestingly, the model was unable to predict the presence of stable cavitation; all bubbles nucleated from the particles go through inertial collapse.

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3 257 These predictions were further verified with high-speed videography of cavitation events from the
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5 258 particles⁶¹. All of the observed cavitation nucleated from nanocups predictably appeared to
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8 259 spherically expand to a maximum radii before collapsing. After collapse, several non-linear
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10 260 phenomenon were also recorded (e.g., jetting, bubble involution, non-spherical shapes, etc.).
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12 261 Bubble detachment from a single nanocup was also observed, giving further validation that
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14 262 bubbles are likely pulled out of the cavities before undergoing expansion and collapse. It was also
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17 263 evident that ultrasound driving frequency governed most of the phenomenon observed, strongly
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19 264 affecting the maximum radii before inertial collapse, closely matching the predicted values⁶².
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21 265 Considering that nanocups produced only inertial cavitation, these events were detected by the
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23 266 presence of an increase of broadband noise from the particles. The clear transition from
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25 267 background noise to the presence of broadband signal from the particles marked the pressure
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27 268 amplitudes necessary for inertial cavitation, i.e., the inertial cavitation threshold. Nanocups with
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30 269 larger diameters were observed to have lower inertial cavitation threshold compared to smaller
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33 270 nanocups, owing to the larger cavity size.
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36 271 Nanocups have also been shown to be capable of improving drug delivery to both tumors and skin.
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38 272 In a xenograft tumor murine model, systemic injection of nanocups with a co-administered drug
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40 273 have shown to increase the transport of the therapeutic agent into a tumor⁵⁸. For the case whereby
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42 274 the drug was an oncolytic virus, the increased oncolytic virus delivery to the tumor led to both a
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44 275 10000 fold increase in the expression of a reporter gene and tumor reduction⁵⁹ (Figure 4B). In the
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47 276 skin, transdermal delivery of vaccines with nanocups was sufficient enough to stimulate an
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49 277 immune response from mice, providing acquired active immunity. The nanocups were shown to
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51 278 not only to enhance the penetration ability of a model vaccine⁶³, but were also observed to
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54 279 penetrate into the skin (Figure 5). In general, the penetration of the nanocups was deeper than the
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280 drug itself, suggesting that loading a therapeutic into the polymeric particle may provide additional
 281 enhancement to the therapy.

283 **Figure 5.** Schema showing ultrasound modal penetration of vaccine transdermally with cavitation
 284 nanoparticle. Reprinted with permission from ⁶³. DOI: 10.1016/j.jconrel.2016.07.016 and copyright
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287 Considering that cavitation was capable of inducing chemical changes to molecules, there was a
 288 concern that inertial cavitation may alter the potency of the co-administered therapeutic, especially
 289 for viral based nanomedicines and antibodies. However, cavitation from nanocups have not yet
 290 been observed to measurably influence the therapeutic efficacy of small drug molecules (e.g.,
 291 doxorubicin), antibodies (e.g., cetuximab), non-enveloped viruses (e.g., adenovirus), and
 292 enveloped viral vectors⁶⁴. Thus nanocups remain a safe and promising solid cavitation agent for
 293 enhanced drug delivery across a variety of biomedical applications.

294 Gold Nanoparticles

Materials such as gold have also been investigated as a suitable candidate for solid cavitation agent due to it being biocompatible and a well-researched material for other therapeutic applications. In order to create a suitable surface for gas trapping, nanocones with a single cavity were produced using an oil-in-water emulsion method. After drying and resuspending the gold nanocones, cavitation was observed with both 0.5 MHz and 1.6 MHz frequencies, though higher pressure amplitudes were required for the higher ultrasound driving frequency (as expected). The cavitation activity from the gold nanocones exhibited only inertial cavitation similar to the polymer nanocups⁶⁵. Cavitation, which enhanced delivery of fluorescent nanobeads from these gold nanocones, was later measured using an agarose gel flow phantom. The fluorescent microscopic images of the agarose phantom with and without exposure to gold nanocones and co-administered fluorescent beads revealed deep (>1 mm) penetration into the agarose gel (Figure 6). A unique plume of fluorescent beads was observed in the opposing direction of the ultrasound source, suggesting that delivery was not only targetable, but may be steered.

Figure 6. (A) TEM of gold nanocone and size measurement by dynamic light scattering (B) Cavitation mediated penetration of gold nanocone when irradiated with ultrasound for 60 seconds. Reprinted with permission from⁶⁵. DOI: 10.1002/adhm.201800184 and copyright 2018 Wiley.

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313 Another gold nanoparticle has also been reported as an acoustic cavitation agent for photothermal
 314 cancer treatment. Here, the surface roughness of the gold nanoparticle acts as the nucleation site
 315 for cavitation. Though the surface roughness reduced the acoustic pressure amplitudes needed for
 316 cavitation, intense pulsed light was also applied to further reduce the cavitation threshold. The
 317 pulsed light created a layer of vapor around the gold nanoparticle due to surface plasmon
 318 resonance, thereby facilitating cavitation. Colon cancer (CT-26) subcutaneous xenograft tumor
 319 mouse models were treated with ultrasound (1.1 MHz, 2W/cm³) for 3 minutes, in addition to
 320 intense pulsed light. This combinational therapy made a demonstrable reduction in tumor volume
 321 with associated improved survival rates compared to control, ultrasound-only, and light
 322 irradiation-only groups⁶⁶. Such an improvement to treatment may be attributed to the improved
 323 penetration and distribution of gold nanoparticles throughout the cancerous tissue (as suggested
 324 by the gold nanocones). With a greater concentration of gold nanoparticles throughout more of the
 325 tumor, light irradiation was capable of heating a greater proportion of cancerous tissue, resulting
 326 in longer survival rates.

327 Until now, all of the designs for solid cavitation agents revolved around a solid core substrate with
 328 multiple pores or a single surface cavity. However, another approach utilizes the aggregation of
 329 gold nanoparticles, which reportedly stabilize a gaseous core (akin to microbubbles). These sub-
 330 micron (~200 nm in diameter) gas-filled gold nanoshells (SAuNPs) nucleate inertial cavitation,
 331 hence propel the gold nanoparticles into the surroundings⁶⁷. These ballistic gold nanoparticles
 332 become embedded into the surrounding tissue, which leave the neighboring cells susceptible to
 333 phototherapy. As such, these particles were developed specifically as a theranostic agent for
 334 cancer. Furthermore, the gold nanoparticles ejected by SAuNPs during ultrasound exposure

aggregated in the acidic conditions of the cancer cells, enhancing the photothermal effect. This aggregation, along with increased delivery into the cells due to cavitation induced sonoporation, decreased the required systemic dose of gold nanoparticles for photothermal therapy compared to previous work (Figure 7).

Figure 7. Delivery of SAuNP in cancer cells by sonoporation (A) Different stages of SAuNP action for diagnostic and therapeutic application (B) a. Deconstruction of SAuNP by ultrasound with aggregation lead peak shift in UV-Vis data b. DLS size measurement and c. Aggregation of SAuNP due to acidic environment shown by TEM imaging. Reprinted with permission⁶⁷. DOI: 10.1039/C8TB00368H and copyright 2018 Royal Society of Chemistry.

Comparisons and Combinations of Solid Cavitation Agents

Each of the designs discussed here have advantages and disadvantages, and have been designed with specific applications in mind. Generally, silica based cavitation agents require low to moderate acoustic intensity to facilitate cavitation at MHz frequencies. As such, much of this work has primarily been investigated for drug release and contrast enhancement. In contrast, there has been extensive work on the ability for nanocups to nucleate cavitation at both sub- and MHz

frequencies. Yet, the acoustic energies require range from moderate to large depending on the size of the nanocup and driving frequency of the ultrasound. Unlike MSNs, nanocups have been developed for their capability to enhance the penetration and distribution of a co-administered therapeutic, and as such drug-loading has not been investigated as of yet. Thus, when MSNs were combined with nanocups, the acoustic pressure amplitudes for cavitation were reduced, and hence enable a deeper and more pronounced drug distribution of drug-loaded MSNs⁴⁶. Gold nanoparticle cavitation agents typically require moderate to intense acoustic power at MHz frequencies for activation. Yet the introduction of a gas pocket on the surface of a cavity, substantially reduced the requisite acoustic energy. Table 1 summarizes all of the current work regarding the cavitation agents discussed in this report. Though each of these studies are typically conducted in isolation, a comparative analysis of microbubbles, nanodroplets, and nanocups for extravasation has been conducted. In this work, nanocups were shown to extravasate deeper at lower acoustic energies compared to the other cavitation agents⁶⁸. All things considered, it is clear and evident that the design of each solid cavitation agent plays a crucial role for enhancing therapy for specific applications.

Conclusions and Future Direction

Acoustic cavitation from ultrasound requires substantial intensities that may indiscriminately damage healthy and diseased tissue. For decades, fluid-based cavitation agents (e.g., microbubbles, phase-change nanodroplet, etc.) have made major headway in the development of ultrasound-enhanced drug and gene delivery to overcome the limitations of ultrasound-initiated cavitation. Within the last decade, solid cavitation agents have emerged as a new means to nucleate cavitation and facilitate contrast enhancement and drug delivery. Solid cavitation agents are an emerging technology, and have shown to be capable of sustaining cavitation, enhancing loaded- or co-

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3 375 administered drug delivery, and extravasation into tissue (for further drug delivery or improved
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5 376 photothermal therapy). However, there remains much to be developed and investigated with solid
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8 377 cavitation agents in order to ensure their success in the lab translates to the clinic. To date, none
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10 378 of the materials used are degradable, which may be a challenge considering the potentially long
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12 379 durations these particles may reside in tissue. Furthermore, additional comparative studies between
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14 380 other cavitation agents are needed. As of now, work on solid cavitation agents has been limited to
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16 381 biomedical field, but may also find utility in sonochemistry. Though there remains much to be
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19 382 done, we look forward to the next advances in this emerging technology.

21
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Type	Design	Synthesis	Application	Model	Intensity	Frequency	Time of irradiation	Result
Beta cyclodextrin capped Mesoporous Silica Nanoparticle (MSN)	Hydrophobic nanovoid	Tetraethyl orthosilicate co-condensation with octyltriethoxysilane	Cavitation induced cancer cell death	ZR75-30 breast cancer cell line and Xenograft mice model of ZR75-30 cancer	1 W cm ⁻²	1 MHz	20 seconds	10% cell viability
Pluronic F127 capped MSN	Hydrophobic nanovoid	Tetraethyl orthosilicate co-condensation with octyltriethoxysilane	Cavitation induced Blood pool imaging	Whole blood	1 W cm ⁻²	1.1 MHz	-	35% signal intensity reduction
MSN with nanocups	Hydrophobic nanovoid and sub micrometre cup	Tetraethyl orthosilicate co-condensation with octyltriethoxysilane	Drug release	-	0.5-4 MPa	0.5 MHz and 1.1 MHz	60 seconds	Significant release of dye

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Bioresorbable janus nanoparticle	Air entrapment n hydrophobic pore via nanostopper technique	Anodized p+ -type silicon wafers made to nanoparticles and applied nanostopper technique for janus configuration	Ultrasound imaging	Gelatin phantom	0.1–0.45 MPa	2.08 MHz	30 seconds	Contrast enhancement ability of J-Psi comparable to SonoVue
Polymer based nanocup	Gas stabilizing cavity	Seed polymerization in polystyrene with deformation inducing Methyl methacrylate/1,2-divinylbenzene coating	Sustained cavitation and distribution of drug	SKOV-3 and CT-26 tumor model	1-1.5 MPa	0.5 MHz	120 seconds	120 seconds of sustained inertial cavitation
Polymer based nanocup + therapeutics	Gas stabilizing cavity	Seed polymerization in polystyrene with deformation inducing Methyl methacrylate/1,2-divinylbenzene coating	Sustained cavitation and drug stability	A549 cells	1.5 MPa	0.5 MHz	10 minutes	No structural and functional changes in therapeutics
Polymer based nanocup + Oncolytic virus	Gas stabilizing cavity	Seed polymerization in polystyrene with deformation inducing Methyl methacrylate/1,2-divinylbenzene coating	Sustained cavitation and viral penetration and gene expression enhancement	SKOV-3, HepG2 and CT-26 tumor model	1.5 MPa	0.5 MHz	>10 minutes	Reduction in tumour by enhanced penetration and function of oncolytic virus
Polymer based nanocup+ hydrogel	Gas stabilizing cavity	Seed polymerization in polystyrene with deformation inducing Methyl methacrylate/1,2-divinylbenzene coating	Transdermal vaccine delivery	Murine transdermal model	1 MPa	.256 MHz	90 seconds	Transdermal Ovalbumin penetration and subsequent immune response
Gold nanocone	Gas stabilizing cavity	Oil-in-water emulsion method	Cavitation induced penetration	MCF-7 cell culture	1.6 – 2 MPa (0.5 MHz) 2.5–3.0 MPa (1.1 MHz)	0.5 MHz 1.6 MHz	-	Sustained cavitation
Gold nanoparticle	Surface roughness and photothermal mediated gas bubble	Sodium borohydride reduction of aqueous tetrachloroauric acid	Gold nanoparticle penetration in tumor by sonoporation	CT-26 tumor model	2W/cm ³	1.1 MHz	3 minutes	Tumour volume reduction and survival rate was improved
Gold nanoparticle	Microbubble stabilized by gold nanoparticles	Citrate mediated reduction of aqueous tetrachloroauric acid and aggregation using pH responsive ligands by electrostatic interaction	Gold nanoparticle penetration in tumor by sonoporation, PAT imaging and Photothermal therapy	U-87 MG xenograft mouse model	50% duty cycle and 1 W cm-2	1 MHz	5 min	Ultrasound mediated sonoporation and PTT mediate tumor reduction with no relapse

Table 1. Summary of existing solid cavitation agents developed for biomedical application.

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621 TOC Graphic

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Figure 1. (A) Air entrapment in hydrophobic pores of P-hMSN. (B) Ultrasound mediated bubble generation in P-hMSN. Reprinted with permission from 44. DOI: 10.1002/adhm.201600030 and copyright 2016 Wiley.

368x392mm (72 x 72 DPI)

Figure 2. Mesoporous silica nanoparticles (MSN) implantation in agarose flow chamber mold at 1.6 MHz and 4 MPa ultrasound parameters. Reprinted with permission from 46. DOI: 10.1016/j.cej.2017.12.051 and copyright 2018 Elsevier.

115x118mm (150 x 150 DPI)

Figure 3. (A) Janus PSi NP fabrication and PEGylation process for ultrasound contrast enhancement. (B) Average intensity plot from B-mode image. Oxidised porous silicon nanoparticle is denoted as O-PSi and Pegylated oxidised porous silicon nanoparticles as PEG-O-PSi. Reprinted with permission from 48. DOI: 10.1021/acsami.7b11007 and copyright 2017 American Chemical Society.

413x185mm (72 x 72 DPI)

Figure 4. Polymeric nanocup as solid cavitation agent. (A) Nanocup synthesis and mechanism of cavitation nucleation (B) HepG2 tumor reduction by systemic co-injection of oncolytic virus and nanocup with intratumor injection of prodrug. Reprinted with permission from 58-59. DOI:10.1002/sml.201501322 and copyright 2015 Wiley. DOI:10.1038/mt.2016.139 and copyright 2016 CellPress. Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

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Figure 5. Schema showing ultrasound modal penetration of vaccine transdermally with cavitation nanoparticle. Reprinted with permission from 63. DOI: 10.1016/j.jconrel.2016.07.016 and copyright 2016 Elsevier. Under creative commons license <https://creativecommons.org/licenses/by/4.0/>.

174x98mm (109 x 100 DPI)

Figure 6. (A) TEM of gold nanocone and size measurement by dynamic light scattering (B) Cavitation mediated penetration of gold nanocone when irradiated with ultrasound for 60 seconds. Reprinted with permission from 65. DOI: 10.1002/adhm.201800184 and copyright 2018 Wiley.

437x260mm (72 x 72 DPI)

Figure 7. Delivery of SAuNp in cancer cells by sonoporation (A) Different stages of SAuNp action for diagnostic and therapeutic application (B) a. Deconstruction of SAuNp by ultrasound with aggregation lead peak shift in UV-Vis data b. DLS size measurement and c. Aggregation of SAuNp due to acidic environment shown by TEM imaging. Reprinted with permission 67. DOI: 10.1039/C8TB00368H and copyright 2018 Royal Society of Chemistry.

488x201mm (72 x 72 DPI)

Emerging Solid Cavitation Agents

TOC

497x200mm (72 x 72 DPI)