

# Sonopermeation to Improve Drug Delivery to Tumors:

## From fundamental understanding to clinical translation

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### Abstract

#### *Introduction*

Ultrasound in combination with microbubbles can make cells and tissues more accessible for drugs and thereby achieve improved therapeutic activity. In this review we establish the term “sonopermeation”, covering mechanisms such as pore formation (sonoporation), opening of tight junctions, stimulated endocytosis/transcytosis, altered perfusion and changes in stromal compartment. Sonopermeation has gained a lot of interest in the last decade, especially for delivering drugs through the otherwise impermeable blood-brain barrier, but also to tumors.

#### *Areas covered*

In this review we summarize various in vitro assays and in vivo setups that have been employed to unravel the fundamental mechanisms involved in ultrasound-enhanced drug delivery, as well as clinical trials that are ongoing in patients with brain, pancreatic, liver and breast cancer. We summarize the basic principles of sonopermeation, describe recent findings obtained in (pre-) clinical trials, and discuss future directions.

#### *Expert Opinion*

We suggest that an improved mechanistic understanding, and microbubbles and ultrasound equipment specialized for drug delivery (and not imaging) are key aspects to create more effective treatment regimens by sonopermeation. Real time feedback and tools to stratify which tumors will benefit from sonopermeation will be important for clinical success.

**Keywords:** Sonopermeation, sonoporation, ultrasound, microbubble, cancer, blood-brain barrier

#### **Highlights:**

- We suggest “sonopermeation” as a new term to describe increased drug delivery by ultrasound and microbubbles.

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- Specialized microbubbles and ultrasound transducers are being developed for therapeutic applications in drug delivery, rather than using combinations of already approved materials.
  - As sonopermeation is being established as one of many treatment options, it will be increasingly important to develop tools to stratify tumors and patient groups, to treat only those who are likely to benefit from such treatment.
  - Real time feedback-based control appears to be a clear step towards safe and effective sonopermeation, and should be applied whenever possible.
  - Understanding the underlying mechanisms and effects of sonopermeation will be crucial to optimize the efficacy and safety to achieve clinical translation.

63 *1. Introduction*

64 Achieving curative treatment of advanced cancer is notoriously difficult and requires that all  
65 cancer cells are killed or inactivated. For advanced cancer, chemotherapy is generally required,  
66 either alone or in combination with other treatment modalities. However, although the drugs  
67 are potent, they are not selective enough and achieving sufficiently high concentrations in  
68 tumors without the occurrence of unacceptable toxic effects is often not possible. Off-target  
69 accumulation can lead to various side effects and limits the doses that can be administered.

70  
71 Nanomedicines, which typically rely on the enhanced permeability and retention (EPR) effect  
72 for improved tumor accumulation, are designed to improve the biodistribution and thereby  
73 therapeutic index of chemotherapeutic drugs [1-3]. Efficiently exploiting the EPR effect in  
74 clinical settings, however, has turned out to be relatively challenging [4-6]. Drugs,  
75 macromolecules and nanoparticles given intravenously face multiple barriers and restrictions  
76 on their way to the target site, complicating efficient delivery. While conventional small  
77 molecule drugs suffer from a large volume of distribution and a rapid renal clearance and hence  
78 relatively low concentrations in the tumor [7, 8], macromolecules and nanoparticles are in  
79 principle restricted to the vasculature, except for areas with inflammation or in tumors, which  
80 are both characterized by leaky blood vessels. According to the EPR-effect, nanomedicines may  
81 extravasate through the hyperpermeable vasculature in tumors where they are retained as a  
82 result of inefficient lymphatic drainage.

83  
84 Multiple features and facts complicate EPR-based tumor targeting. For instance, the vasculature  
85 in tumors is often highly irregular and chaotic, leaving parts of tumors very poorly perfused [9-  
86 11]. In addition, the leakiness of the blood vessels tends to be highly heterogeneous [12-14].  
87 After extravasation, the penetration of drug carriers is restricted by the presence of dense  
88 stroma [15], the high interstitial fluid pressure observed in many tumors [16-19], and the cell  
89 membrane of the tumor cells. Together, these barriers make it very challenging to achieve  
90 sufficient degree of targeted drug delivery, especially to the deeper parts of the tumor,  
91 precluding curative drug therapy [20, 21]. In the brain, drug delivery is particularly complicated  
92 by the blood-brain barrier (BBB), which is formed by endothelial cells and pericytes lining the  
93 brain capillaries, connected by tight junctions to protect the brain from potentially harmful  
94 blood-borne molecules and materials [22].

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96 Based on these limitations, multiple research groups and pharmaceutical companies are  
97 developing methods that can increase the tumor accumulation and cellular penetration of drugs  
98 and drug delivery systems [23, 24]. Studies are ongoing to address whether this can be achieved  
99 either by administering agents such as vasodilators, blood vessel normalizing agents or  
100 molecules that modulate the extracellular matrix, by the use of stimuli-responsive nanocarriers  
101 reacting to specific features associated with the target disease (such as enzymes, redox potential  
102 or changes in pH), or nanocarriers responsive to locally applied external triggers (such as light,  
103 temperature, magnetic fields or ultrasound) [23, 25-27].

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105 *2. Ultrasound in drug delivery*

106 Ultrasound in medical diagnostics is a safe and widely applied real-time imaging modality.  
107 During the last decades, ultrasound has also been increasingly studied for therapeutic purposes  
108 [28-30]. Because it can be focused, it can be used to deliver energy to small volumes deep inside  
109 the body without affecting intermediate tissues. Ultrasound is generally non-invasive and  
110 localized and can, depending on the desired application, be tuned to create thermal effects such  
111 as heating, or mechanical effects such as acoustic radiation force or acoustic cavitation [31, 32].  
112 When ultrasound waves pass through tissue, the waves will be attenuated by scattering and by  
113 absorption [33]. The absorption of acoustic energy by tissue causes thermal heating [32-34].  
114 High intensities can be employed to create hyperthermia for applications in physiotherapy [35]  
115 and tissue ablation with real time temperature mapping (via magnetic resonance imaging; MRI)  
116 [36, 37]. Local mild hyperthermia can also be used to increase drug release from nanocarriers

117 such as thermosensitive liposomes [38, 39], and to locally increase blood flow [40, 41], vascular  
118 permeability [42, 43], diffusion of drugs, and possibly cellular uptake [31, 44], thereby  
119 enhancing delivery of therapeutic agents. A radiation force [45] in the direction of wave  
120 propagation is caused by a momentum transfer from the ultrasound wave to the transmitting  
121 medium [32]. This force can produce a steady flow in fluids (known as acoustic streaming), and  
122 may therefore potentially increase convective transport [46]. It could also cause local tissue  
123 displacements [33] and disrupt extracellular matrix for increased extravasation and interstitial  
124 penetration [32]. In addition, acoustic radiation forces have been reported to modulate the  
125 direction and velocity of flow of ultrasound contrast agents, i.e. microbubbles, for instance by  
126 pushing them towards the vascular wall while they circulate in tumor blood vessels [45].

127  
128 The use of ultrasound in the presence of exogeneous gas bubbles can lead to cavitation and local  
129 forces strong enough to cause membrane permeabilization. Cavitation refers to the creation  
130 and/or oscillation of gas bubbles upon exposure to an acoustic field, in response to the  
131 oscillating acoustic pressure [31, 34]. By the use of ultrasound and microbubbles, improved  
132 effect of conventional chemotherapeutics has been demonstrated in patients with non-  
133 resectable pancreatic tumors (PDAC) [47, 48] and in clinical trials with glioblastoma patients  
134 (table 1) [49]. Preclinically, the effect has been evaluated for a myriad of indications. As there  
135 are multiple excellent reviews on the topic [30, 50-54], we here focus on how these effects are  
136 frequently explained, review some systems created specifically for drug delivery, and suggest  
137 future directions to improve tumor-targeted drug delivery and achieve clinical impact.

### 138 139 *2.1. Sonopermeation*

140 This review presents studies demonstrating increased drug delivery by ultrasound and  
141 microbubbles regardless of underlying mechanisms. The term sonoporation has often used to  
142 describe these mechanisms [51, 52]. However, the term sonoporation refers to the formation of  
143 'pores' by the use of sound, which is only a subset of the effects that have been shown for  
144 ultrasound and microbubbles. In this review we establish the term "sonopermeation" as a term  
145 describing increased therapeutic effect achieved by ultrasound and microbubbles. We suggest  
146 that the term "sonoporation" will be used specifically for the formation of pores. Sonopermeation  
147 describes the non-thermal and mechanical effects achieved with the combination of ultrasound  
148 and exogenous microbubbles. It is hypothesized to function both through the formation of  
149 transient pores in cell membranes (sonoporation), the opening of intercellular (tight) junctions  
150 [51, 55-57], stimulated/altered endocytosis, transcytosis or exocytosis [58, 59], macroscopic  
151 changes in perfusion [60] and changes in extravascular, and perivascular space [61]. As  
152 pressure waves pass through tissues, microbubbles in the pressure field will expand at low  
153 pressures (rarefaction) and contract at high pressures (compression), creating volumetric  
154 oscillations in phase with the applied ultrasound [34]. Stable cavitation occurs at relatively low  
155 amplitudes, and is characterized by sustained bubble radius oscillation about its equilibrium  
156 [32]. These oscillations can be detected as harmonic signals from the microbubbles. Oscillating  
157 microbubbles will generate a circulating fluid flow, known as microstreaming, which has  
158 velocities and shear rates proportional to the amplitude of oscillation [31, 62, 63] and to the  
159 applied pressure. If the microbubbles are close to the endothelium, they can also push and pull  
160 on the cell membrane [64], and especially the pulling motion, creating elongation of the cell  
161 membrane has been suggested to induce formation of pores [65]. Inertial cavitation occurs  
162 when larger amplitude oscillations result from an increased acoustic pressure [31]. The  
163 amplitude of oscillation increases until the inrushing fluid has sufficient inertia to overcome the  
164 internal pressure of the bubble, and then the bubble will collapse [31, 34]. The extreme  
165 compression of the gas by the liquid creates high pressures and high temperatures, and the  
166 fragmentation of the microbubble results in smaller bubbles which can again cavitate, grow and  
167 collapse [31]. Following the collapse of a bubble, shock waves are created and liquid jets can  
168 occur if the bubble collapses near a surface [31, 32, 51]. The oscillation and collapse of  
169 microbubbles can also cause formation of free radicals [51], leading to cytotoxicity and  
170 potentially cell death [44].

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## 2.2. *Ultrasound parameters for sonopermeation*

To enable controlled drug delivery without causing tissue damage, careful control of ultrasound parameters is required. For sonopermeation, the ultrasound wave is typically pulsed both to avoid tissue damage from heating and to allow for inflow of microbubbles between the pulses in cases where bubble destruction is expected. The sinusoidal ultrasound wave is often described in terms of its velocity, wavelength, frequency (or period), pressure amplitude, pulse length (or burst duration), pulse repetition frequency (PRF), total exposure time (or duty cycle) and total treatment time [33, 66, 67]. Mechanical index (MI), defined as the peak negative pressure amplitude (MPa) divided by the square root of the center frequency (MHz) of the transmitted ultrasound wave, is often used to classify microbubble behavior, and the probability of inertial cavitation occurring increases with increasing MI [31, 64]. Frequently used parameters for sonopermeation and drug delivery vary greatly between different studies, including frequencies of 0.5-3 MHz with pressures of 0.05-2 MPa, pulse lengths of 2-10 000 cycles with a PRF of 0.25 Hz - 10 kHz, and total exposure times of seconds to hours with duty cycles varying from less than 1% to 50% [52, 66, 68-73]. The response of a microbubble will depend highly on the ultrasound settings [52, 64]. Increasing the pressure, sonication time, burst length or pulse repetition frequency has been shown to give increased permeability of vasculature in the brain [74]. It has also been suggested that higher pressures and thus larger oscillations and a more violent collapse probably induces larger pores, which are required to deliver nanoparticles and gene complexes compared to low molecular weight drugs [51]. By applying real-time feedback of acoustic emission from the microbubbles, the ultrasound parameters can be standardized to the microbubble response in each animal [75, 76]. By doing this, it is possible to eliminate in situ pressure fluctuations caused by variations in tissue absorption of ultrasound, variations in skull thickness when intending to open the blood-brain barrier, or differences in bubble concentration caused by varying vascularization and perfusion between tumors. The harmonic signal may then be used to monitor bubble behavior, with subharmonic and ultraharmonic emissions indicating stable cavitation [77-79], and increased broadband acoustic emission indicating bubble destruction or inertial cavitation [80, 81].

## 2.3. *Biological effects of sonopermeation*

Various methods have been reported in the literature to study the mechanisms and effects of bubble-cell interactions [50, 82]. Some examples of how oscillating microbubbles can interact with cells are illustrated in Figure 1. The resulting streaming and shear forces, and/or push-pull-effects on the vessel wall induced by stable cavitation, can cause formation of small pores for increased vascular permeability, and they can also enhance endocytosis which can contribute to transfer of drugs over the membrane [51, 52, 58, 64, 83]. Following the collapse of a bubble, the resulting shock waves and liquid jets can create both temporary and permanent pores in the capillary wall and in cell membranes [31, 32, 51]. Various pore sizes are reported in the literature, from a few nanometers to several hundreds of nanometers, and even larger [84-88]. Membrane integrity is vital for cell survival, hence membrane wound healing processes will quickly start repairing the membrane after sonoporation [89]. Hu et al. investigated the dynamics of pore formation and resealing, and determined which pore sizes are non-resealable [88].

Focused ultrasound has been used to deliver molecules to and into cells in vitro by sonoporation [51, 65, 88], which has also been demonstrated in vivo in endothelial cells [90]. It has been shown that sonopermeation can be employed to increase extravasation across the capillary wall and potentially improve penetration through the interstitium, thereby improving the accumulation and distribution of drugs and drug delivery systems in solid tumors [91-97]. Similar mechanisms have been suggested to be involved in sonopermeation-based BBB-disruption for drug delivery to the brain [66, 98, 99]. Upon sonication, microbubble oscillations will exert mechanical stress on the endothelial cells and their tight junctions, possibly

225 generating a paracellular transport route [57, 99, 100]. It has also been suggested that  
226 transcytosis can be induced by ultrasound [58, 99, 101, 102], and that transient formation of  
227 fenestrations in the endothelial cell membrane can contribute to transcellular transport [58,  
228 99]. Additionally, ultrasound combined with microbubbles has been reported to down-regulate  
229 the expression of drug efflux pumps (such as P-glycoprotein) in endothelial cells in the brain  
230 [103, 104]. By inhibiting drug efflux, the accumulation and retention time of drugs in the brain  
231 can be increased. Also, oscillating microbubbles can increase penetration of drugs through the  
232 brain parenchyma by the perivascular pump-effect, explained by increased arterial pulsation  
233 [105, 106].

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235 Another likely (but less explored) effect of sonopermeation is altered perfusion (Figure 1).  
236 Ultrasound and microbubbles have been shown to cause a vasoconstriction or vascular shut  
237 down, and reduced perfusion in tumors, brain and other tissues [107-110]. This has also been  
238 used in a synergistic manner in combination with radiation therapy [111]. In contrast, locally  
239 increased perfusion has also been reported [112]. In a study on repetitive ultrasound exposures,  
240 Rix and coworkers found increased peak signal enhancement in tumors after repetitive  
241 microbubble injections and speculated that among other reasons, this might be due to the  
242 mechanical opening of non-perfused microvessels [60].

#### 243 244 *2.4. Microbubble platforms and ultrasound transducers*

245 Sonopermeation as a research field is rapidly expanding, and specialized equipment for  
246 therapeutic ultrasound procedures is emerging and steadily evolving. The microbubbles which  
247 are typically used for this application are ultrasound contrast agents with sizes of 1-10  $\mu\text{m}$ , thus  
248 restricting them to the vascular compartment [113]. Commercial microbubble formulations  
249 have been used for decades in the clinic to enhance echogenicity of blood in diagnostic  
250 ultrasound [33]. Various types are commercially available with shells of either protein  
251 (Optison®) or lipids (SonoVue®, Sonazoid®, Definity®). They contain heavy gases instead of  
252 air for increased stability, which is excreted by exhalation, whereas the shell is excreted by the  
253 reticuloendothelial system in liver and spleen (RES) [113]. They can be used with a co-  
254 administration of a drug, or the drug may be loaded into or onto the bubbles in various ways  
255 [52, 53, 64, 114, 115]. Microbubbles may also be targeted to molecular markers expressed on  
256 endothelium of specific diseases [52, 116]. The response of a microbubble to ultrasound  
257 depends highly on properties of the microbubble such as size, shell thickness and stiffness [51,  
258 64], and the largest oscillation response of microbubbles is obtained at their resonance  
259 frequency, which decreases with increasing size [64]. The majority of studies performed to date  
260 (and all clinical trials) are performed with conventional soft-shell microbubbles that are  
261 tailored for imaging purposes. These microbubbles are well characterized and approved in the  
262 clinic, but it has been shown that the effect of polymeric hard-shell microbubbles can be greater  
263 in some situations [117] and that both transfection and nanoparticle delivery by sonoporation is  
264 more effective if the nucleic acid or nanoparticle is attached to the microbubble [118, 119].  
265 Sonopermeation has been shown using a multitude of microbubbles such as nanoparticle-  
266 loaded [92, 119, 120] or even nanoparticle-stabilized microbubbles [121], hard-shelled  
267 microbubbles [122], and clusters of microbubbles and emulsions of liquid perfluorocarbons that  
268 change phase and expand upon insonification [91]. Other systems have also been suggested,  
269 such as nanodroplets which can be activated in the interstitium [123] and antibubbles where  
270 the microbubbles contain a liquid droplet [124]. In general, there is a lack of systematic studies  
271 comparing the effect of different microbubbles for drug delivery applications [79]. These studies  
272 would also be challenging, as the various microbubble constructs will likely require different  
273 ultrasound settings for optimal effect.

274  
275 For ultrasound platforms, a lot of the early work was done using clinical imaging systems. The  
276 advantage is the combination of both imaging and drug delivery simultaneously, however the  
277 range of ultrasound parameters available is limited. Gradually, and especially for BBB-  
278 applications, there has been a development of more specialized equipment using far lower

279 frequencies compared to diagnostic ultrasound imaging. In clinical trials on glioma, two very  
280 different approaches have been suggested, either implanting the ultrasound device inside the  
281 skull (SonoCloud®)[49], or image-guided sonication through the skull from multiple angles to  
282 obtain sufficient pressures at the focal spot (Exablate Neuro®)[125]. Other systems have been  
283 developed for ultrasound treatment elsewhere than the brain (Sonablate®, Insightec  
284 ExAblate® and Sonalleve®). In the clinical trial on pancreatic adenocarcinoma, an unmodified  
285 diagnostic ultrasound scanner was used in combination with lipid microbubbles [47].

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### 287 *2.5. In vitro models to study sonopermeation*

288 Several different in vitro models are being employed to investigate the fundamental biological  
289 and biophysical processes involved in sonopermeation. Various types of cells, grown as  
290 monolayers or cells in suspension, are used to gain insights in microbubble-cell interactions and  
291 how the oscillation dynamics affect the cell membrane and transport of model drugs [50, 88,  
292 120, 126-128]. It is unclear how well these assays mimic the in vivo situation and more complex  
293 and physiologically relevant models have been designed. 3D models such as cell  
294 clusters/spheroids [129], organs-on-chip including vessels [130], ECM components and co-  
295 cultures of various cells [131, 132], excised tissues [133, 134], or the chicken embryo model  
296 [90] can also be used. Different types of instrumentation have been employed to obtain  
297 complementary information on the time- and length-scales of the involved phenomena, as  
298 summarized by Lajoie et al. [50]. Much of the knowledge of microbubble dynamics and the  
299 impact on cells upon sonication comes from optical imaging, with fluorescence imaging and  
300 high-speed imaging most commonly used [65, 88, 135]. However, also electron microscopy,  
301 atomic force microscopy, confocal microscopy and flow cytometry have been used to evaluate  
302 perforations in the cell membrane [50, 88, 136-138]. It has been shown that sonoporation can  
303 create holes in the cell membrane, both destructively and reversibly [88, 89, 136, 139] and also  
304 that tight junctions can be opened [140]. It has been demonstrated that a close contact between  
305 the cell and the microbubble is needed [65] and that a certain vibration amplitude of the bubble  
306 is necessary for pore formation [127, 141]. Sonoporation has also been used for in vitro  
307 transfection of dendritic cells, to achieve a therapeutic effect upon re-injection of the dendritic  
308 cells [84] and subsequent studies have indicated that such transfections also can be performed  
309 in vivo [142].

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### 311 *2.6. Sonopermeation of tumors*

312 The potential of sonopermeation for delivery of free or encapsulated chemotherapeutics to solid  
313 tumors has been demonstrated in several preclinical studies and summarized in reviews [52,  
314 53]. It has been shown that sonopermeation can increase delivery of both drugs and  
315 nanoparticles giving reduced tumor growth and in some cases even curative therapy (Table 1.)  
316 Perhaps due to less challenging experimental setups, tumor models outside the brain have been  
317 used to test novel microbubbles not yet approved for clinical use. There are multiple studies  
318 showing that drugs and drug delivery systems loaded onto microbubbles can have improved  
319 antitumor effects compared to co-injection regimens (Table 1). This supports the notion that  
320 increased effect of sonopermeation can be anticipated as more specialized systems are tested in  
321 clinical trials. Another novel concept is the injection of microbubble-microdroplet clusters that  
322 will undergo a phase shift upon ultrasound, creating large bubbles that temporarily deposit in  
323 and block capillaries. This system was used in combination with Abraxane® to successfully cure  
324 the majority of prostate tumor-bearing mice [91]. Interestingly, in the same study, microbubbles  
325 alone (as opposed to the clusters) were found to severely reduce the effect of Abraxane®,  
326 possibly due to decreased perfusion of the tumor obtained by the selected ultrasound settings  
327 and microbubble type.

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329 The only reported clinical trial to date using sonopermeation to treat solid tumors evaluated the  
330 safety and potential toxicity of combining gemcitabine with microbubbles under sonication in  
331 ten inoperable pancreatic cancer patients [47]. Dimcevski and colleagues reported that the  
332 combination of clinically available ultrasound equipment with commercial microbubbles and

333 chemotherapy resulted in no additional toxicities. Furthermore, the combined treatment  
334 enhanced the clinical efficacy of gemcitabine and extended survival in patients with pancreatic  
335 adenocarcinoma. Several similar studies have been initiated in patients suffering from breast  
336 cancer, liver metastasis resulting from primary colon cancer, and pancreatic cancer (Table 1).

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### 338 2.7. Sonopermeation of the blood brain barrier

339 Sonopermeation of the blood brain barrier to access brain tumors is one of the most developed  
340 and promising applications of therapeutic ultrasound [66, 143]. The vasculature and biological  
341 barriers faced by drugs in the brain and in brain tumors are somewhat different from those in  
342 tumors located elsewhere, the BBB with its tight junctions and high density of efflux pumps is a  
343 formidable barrier for drug delivery to the brain. Following the first demonstration of reversible  
344 BBB-opening by ultrasound in rabbits [68], there have been extensive efforts in further  
345 developing the concept in pre-clinical settings [66, 144, 145]. Successful BBB-opening, increased  
346 delivery and/or improved therapeutic efficacy have been demonstrated for chemotherapeutic  
347 drugs [54, 146], nanoparticles [147-149], antibodies [150-152], interleukins [153] and cells for  
348 immunotherapy [154, 155]. Safety has been evaluated in both small animals and in non-human  
349 primates, and no adverse effects were observed in awake and behaving primates [156, 157]. It  
350 has also been shown by multiple groups that the BBB-opening is temporary and is reversed  
351 within minutes to hours and that the window for drug delivery to the brain depends on the size  
352 of the drug/nanocarrier [147, 158, 159]. The procedure is generally considered to be relatively  
353 safe, but this consensus was recently challenged following the work by Kovacs et al. who  
354 showed that BBB-opening could induce a local inflammation [160-162] and suggested that the  
355 procedure should be evaluated in more depth before going into clinical practice. Even though  
356 small extravasations and mild inflammatory reactions have been observed in the sonicated area  
357 by some, ultrasound in conjunction with microbubbles was not reported to result in damage of  
358 neurons, neither directly, nor through ischemia or apoptosis, nor by delayed effects up to one  
359 month after sonication [163]. One method to increase both the efficacy and the safety of BBB-  
360 opening is through real-time feedback of *in situ* sonopermeation, which will reduce the effects of  
361 variations in microbubble concentration and ultrasound attenuation. It was recently shown that  
362 feedback control through the detection of harmonics from the microbubbles could be used to  
363 precisely control the magnitude of the BBB-opening and the amount of drug delivered to the  
364 brain [164]. Clinically, the development is being fronted by groups in France and Canada  
365 pioneering the development of SonoCloud®, an implantable ultrasound transducer, and  
366 ExAblate Neuro®, an image guided transcranial array of transducers, respectively (Table 1).  
367 The phase I trial with SonoCloud® reported no adverse effects and it did provide initial  
368 indications for therapeutic responses [49].

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370 Besides brain tumors, BBB-opening is also being evaluated for the treatment of other diseases  
371 in the brain. Promising results have e.g. been achieved in preclinical models of Alzheimer's  
372 disease [75, 165-167] and Huntington's disease [168], as well as in a Parkinson's disease mouse  
373 model via the delivery of neurotrophic factors [169]. Furthermore, ultrasound-mediated  
374 delivery appears promising for stem cell delivery/treatment [170], for the delivery of viral  
375 vectors and gene therapy [171-173], and for the treatment of stroke [174].

376

### 377 3. Conclusion

378 From pioneering achievements in the last decade using materials and methods intended for  
379 imaging, the development is now going in the direction of more specialized systems to achieve  
380 maximum, but controlled drug delivery. Targeted drug delivery by sonopermeation is  
381 progressing rapidly towards clinical practice; the first clinical trials on BBB opening and  
382 treatment of patients with pancreatic cancer have been finalized, and multiple clinical trials  
383 with sonopermeation of solid tumors are recruiting. Although our understanding of both  
384 mechanisms and adverse effects is still incomplete, the strong pre-clinical evidence and the  
385 positive outcome of the performed clinical trials suggest that sonopermeation is a promising  
386 approach for treatment of tumors and neurodegenerative disorders.



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#### 4. Expert Opinion

Sonopermeation is a technology that is rapidly moving towards clinical practice, based on promising results obtained in proof-of-principle studies in animal models. Multiple clinical trials are currently ongoing, of which the vast majority are exploiting combinations of clinically approved microbubbles and drugs. While it is sensible to break new ground with established methods combining already approved components, the development is now going in the direction of more specialized systems, produced especially for drug delivery. It has been demonstrated pre-clinically that microbubbles developed for therapy can be superior to the clinically approved alternatives, tailored for imaging applications. In addition, many pre-clinical experiments involve ultrasound settings outside the range of diagnostic ultrasound scanners, indicating a need for developing transducers specialized for therapeutic applications. On the other side, there are obviously very appealing advantages associated with the use of systems that are already approved as the road to clinical use is much shorter both financially and regulatory.

Despite the promising results obtained so far, the field is still lacking a complete understanding and explanation of some of the observed effects. The currently most frequent explanation is transient pore formation in the cell membrane or opening of cell junctions, but neither of these are completely described or understood at a microscopic level. These are two distinct mechanisms with different consequences (i.e. intracellular vs. extracellular delivery) and should be evaluated and possibly exploited selectively. However, observations not easily explained by this theory are sometimes encountered. One example is the improved effect of gemcitabine after sonication [47]. Gemcitabine is a small water-soluble molecule that should be able to cross endothelial membranes and diffuse through tissue efficiently. The mechanism is not elaborated in the paper, but it seems plausible that increased perfusion and vessel decompression, in addition to permeabilization of the blood vessel wall, contributed to the enhanced efficacy of gemcitabine. Another example is the detrimental effect of sonopermeation with Sonazoid® on the effect of Abraxane® as seen in a subcutaneous prostate cancer model in mice [91]. Here, the therapeutic effect of Abraxane® was lost if the drug was combined with lipid microbubbles, but greatly improved when combined with the microbubble-microdroplet clusters. The unexpected effect with Sonazoid® could not be further explained based on the study's results. It may be the result of decreased perfusion of the tumor obtained by the selected ultrasound settings.

While a complete understanding is not a prerequisite for clinical success, sonopermeation has almost endless degrees of freedom. Finding the most effective combination of drug, drug delivery vehicle/formulation, microbubbles and ultrasound settings, as well as dosing and treatment schedule through “trial-and-error” seems unrealistic, especially when considering that different diseases require different treatment regimens. Sonopermeation has been proven effective for different types of solid tumors, brain tumors, as well as neurodegenerative disorders, each of which has its own characteristic barriers for drug delivery and hence the potentiating effect from sonoporation differs in these cases. As the toolbox of drug delivery materials and methods expands, it will be increasingly important to develop an understanding of which patients will actually benefit from a specific approach. As sonopermeation is established as one of many treatment options, tools to stratify patient groups, such as magnetic resonance or ultrasound imaging or disease-specific molecular biomarkers, will be needed. However, achieving personalized treatment, tailored treatment regimens and real-time feedback control for sonopermeation requires a better understanding of the (bio) mechanics involved.

In terms of understanding, we are closer to elucidating the mechanism of action for ultrasound-mediated BBB-opening. Increased permeability of the otherwise tightly controlled blood vessel wall has made it possible to deliver drugs to the brain and will likely also increase the drive for development of new drugs for diseases in the brain. The results from clinical trials in France and

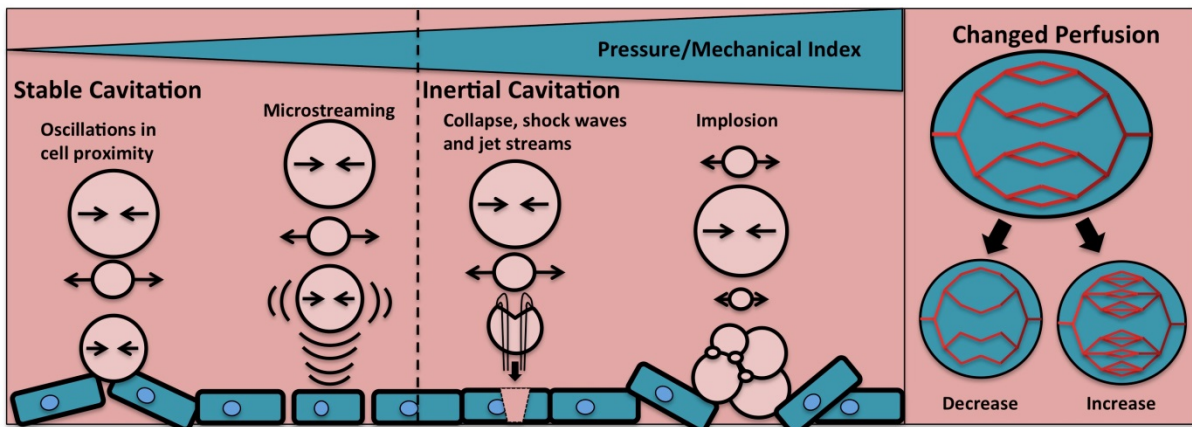
441 Canada will shed light on the possible clinical effects and the strengths/weaknesses of these two  
 442 different setups. Also the development of feedback-based control - which has been ongoing [76]  
 443 and which has been recently demonstrated [164, 175] - is a clear step forward in the direction  
 444 of control and understanding of sonopermeation-based BBB opening.

445  
 446 Specific focus on the limitations in the current application of sonopermeation is needed to  
 447 produce more effective therapeutic solutions. In Figure 2, we have highlighted four studies that  
 448 exemplify what should be focus areas in order to advance sonopermeation. Understanding the  
 449 involved mechanisms and relation to the different biophysical effects will be crucial to optimize  
 450 the efficacy and safety for ultrasound-mediated drug delivery and achieve translation to clinical  
 451 benefit (panel 1). Also, indications from pre-clinical research with specialized microbubbles  
 452 (panel 2) and equipment (panel 3) has shown that therapy-specific setups can be superior  
 453 compared to combinations of already approved materials. Furthermore, while disease models  
 454 are invaluable tools in medical technology, the real therapeutic potential of sonopermeation can  
 455 only be evaluated in clinical trials (panel 4), especially as the ultrasound equipment and relative  
 456 doses of microbubbles used in pre-clinical research in rodents often is not translatable.

457  
 458 Even though ultrasound can be used for both superficial and deep tumors with imaging  
 459 guidance, sonopermeation has the limitation of being site-specific, which implies that only  
 460 tumors with known location can be treated. However, the abscopal effect, which can sometimes  
 461 be observed after radiation treatment, has shown that localized therapies can have systemic  
 462 effects [176]. In case of the abscopal effect, local treatment can have systemic consequences as  
 463 a result of shedding of tumor antigens from the treated region, thereby priming the immune  
 464 system towards a response (especially when combined with e.g. anti-PD(L)1 and anti-CTLA4  
 465 immunotherapies) [177]. As soon as exploitation of the abscopal effect becomes fully  
 466 understood and a clinical reality, sonopermeation could be an important tool also for the  
 467 treatment of advanced metastatic cancers.

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 469 In our opinion, sonopermeation is developing in a promising manner through collaborative  
 470 efforts in the field of ultrasound physics, chemistry, pharmacy, biology and medicine. We still  
 471 have quite a way to go in terms of fundamental understanding, and this may be the limiting step  
 472 in the development of more disease-specific setups. However, as the results from clinical trials  
 473 with specialized materials and methods are becoming available, and as more refined systems  
 474 are being evaluated, we expect the outcomes to be gradually improving. Improved outcomes  
 475 will generate increased interest and funding, which will eventually lead to specifically  
 476 developed and properly understood setups that can be applied to a stratified group of patients,  
 477 resulting in prolonged survival times and improved quality-of-life.

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 481 Figure 1: Schematic illustration of possible vascular effects of sonoporation on the capillary wall  
 482 and on perfusion.

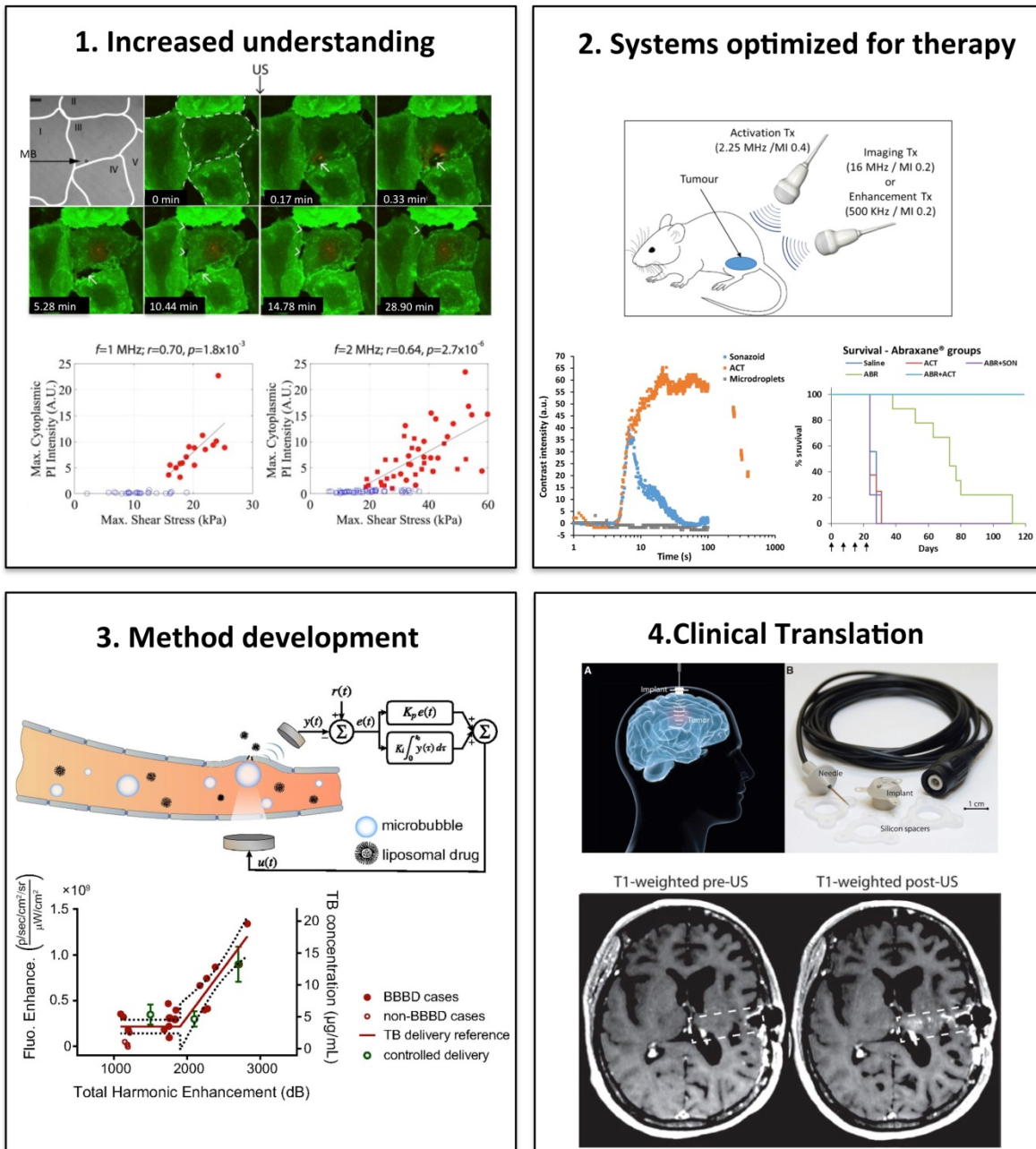
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Table 1: Therapeutic studies using sonopermeation.

<b>Selected Preclinical Studies</b>				
<b>Target</b>	<b>Drug</b>	<b>Setup</b>	<b>Results</b>	<b>Ref.</b>
Dendritic cells	mRNA	mRNA-lipoplex-loaded microbubbles, 0.8 MPa in Opticells®	Therapeutic effect in two tumor models, no tumor upon rechallenge	[84]
PC3 prostate adenocarcinoma	Paclitaxel / Abraxane®	ACT® 2.25MHz activation, 0.5MHz enhancement	Combined with Abraxane®, complete remission in 6/9 tumors	[91]
Ca9-22 gingival squamous cell carcinoma	Bleomycin	Microbubbles targeted with EGFR-antibodies injected directly into tumor, 1 MHz	Growth inhibition of all 4 tumors only when microbubbles are targeted	[178]
C6 glioma	5FU-loaded nanoparticles	Albumin microbubbles with 5FU loaded nanoparticles attached to the surface, 1MHz, 1.2 MPa	5x increased tumor accumulation compared to without ultrasound, significantly improved therapeutic effect	[92]
MIA PaCa-1, pancreatic adenocarcinoma	Gemcitabine	Lipid microbubbles, 1MHz, MI=0.2	Reduced tumor volume, but not significantly increased survival with ultrasound	[93]
CT-26 colorectal adenocarcinoma	Pegylated liposomal doxorubicin (Doxil®)	Lipid microbubbles, 1MHz	Increased accumulation of doxorubicin in tumors and improved therapeutic effect	[95]
C6 glioma	VEGF-targeted and carmustine-loaded microbubbles	In-house lipid microbubbles, 1 MHz, 0.5MPa	Enhanced local delivery of chemotherapeutic agent, reduced tumor progression and improved median survival time	[179]
9L gliosarcoma	Liposomal doxorubicin	Lipid microbubbles, 1.7 MHz	Reduced tumor growth and improved survival	[148]
4T1 breast carcinoma	Paclitaxel-liposome-microbubble complexes	2.25 MHz	Inhibited tumor growth	[180]
MDA-MB-231 breast carcinoma	Cabazitaxel-loaded nanoparticles	Nanoparticle-stabilized microbubbles, 1MHz	Complete remission in 3 / 3 tumors	[121]
Glioblastoma multiforme	Doxorubicin	PEGylated lipid microbubbles 612.5 kHz	Increased doxorubicin concentration, increased survival and slower disease progression	[181]
MCF-7 breast	Doxorubicin prodrug	Prodrug-microbubble complex, 1 MHz	Higher tumor inhibition rates	[182]
<b>Clinical Trials</b>				
<b>Target</b>	<b>Deliverable</b>	<b>Setup</b>	<b>Goal/Results</b>	<b>Ref.</b>
Glioma	Carboplatin	Implantable	Safe BBB-opening above	[49]

		ultrasound transducer, SonoCloud®	0.8MPa	NCT02253212
Pancreatic cancer	Gemcitabine	Diagnostic ultrasound scanner, linear probe MI=0.2	Doubled median survival (from 8.9 to 17.6 months)	[47]
Hepatocellular carcinoma	Yttrium-90 loaded microspheres	Albumin microbubbles and diagnostic ultrasound	Currently recruiting	NCT03199274
Glioblastoma	-	ExAblate® BBB-distruption prior to surgery	Assess safety and feasibility of BBB-opening in patients undergoing surgery	NCT03322813
Breast cancer	Neoadjuvant epirubicin, cyclophosphamide, paclitaxel, carboplatin	Lipid microbubbles, diagnostic ultrasound scanner, linear probe, high MI	Assess increase in tumor perfusion after sonoporation and response to neoadjuvant chemotherapy	NCT03385200
Hepatic metastases from colorectal cancer	FOLFIRI plus bevacizumab	Lipid microbubbles combined with ultrasound	Assess safety and tolerance, decreased tumor size and assessment of vascularity	NCT03458975
Liver metastases from gastrointestinal tumors and pancreatic carcinoma	Oxaliplatin with paclitaxel and gemcitabine.	Lipid microbubbles combined with ultrasound	Assess safety and efficacy	NCT02233205
Brain tumors	Liposomal Doxorubicin or Temozolomide	Transcranial ExAblate®	Demonstrated safety of BBB-disruption using transcranial MRI-guided focused ultrasound	[183] NCT02343991
Liver metastases from breast cancer and colorectal cancer	Paclitaxel or FOLFIRI	Lipid microbubbles with ultrasound	Difference in response between ultrasound-treated and untreated lesions	NCT03477019

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489 *Figure 2: Examples of studies advancing the use of sonopermeation. 1: Helfield et al. demonstrated*  
 490 *that sonoporation initially creates a transient hole in the cell membrane allowing for intracellular*  
 491 *drug delivery. Subsequently, pores are formed between the endothelial cells possibly creating the*  
 492 *basis for BBB-opening and drug extravasation. Figure adapted from [127]. 2: van Wamel et al.*  
 493 *demonstrated that acoustic cluster therapy (ACT®) could overcome some of the limitations of*  
 494 *standard microbubbles (small size limiting contact with the vessel wall, and short circulation*  
 495 *lifetime limiting exposure time), and hence increase the potential for acoustic effects significantly*  
 496 *and potentiate Abraxane® for the successful treatment of a prostate cancer model in mice. Figure*  
 497 *adapted from [91, 184] with permission from Elsevier. 3: Sun et al. designed a setup for BBB-*  
 498 *disruption where feedback from the harmonic signal from stable cavitation was used to control the*  
 499 *ultrasound pressure and also the amount of drug delivered to the brain. Figure adapted from*  
 500 *[164]. 4: Carpentier et al. demonstrated in a clinical study that the BBB could be safely opened in*  
 501 *glioma patients using an implanted ultrasound transducer (SonoCloud®). Figure adapted from*  
 502 *[49] with permission from The American Association for the Advancement of Science.*

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## Conflict of interest

The authors declare no conflict of interest.

## References

- [1] T. Lammers, F. Kiessling, W.E. Hennink, G. Storm, Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress, *J Control Release*, 161 (2012) 175-187.
- [2] J.J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities, *Nat Rev Cancer*, 17 (2017) 20-37.
- [3] D. Peer, J.M. Karp, S. Hong, O.C. Farokhzad, R. Margalit, R. Langer, Nanocarriers as an emerging platform for cancer therapy, *Nat Nanotechnol*, 2 (2007) 751-760.
- [4] V.J. Venditto, F.C. Szoka, Cancer nanomedicines: So many papers and so few drugs!, *Adv Drug Deliver Rev*, 65 (2013) 80-88.
- [5] R. van der Meel, T. Lammers, W.E. Hennink, Cancer nanomedicines: oversold or underappreciated?, *Expert Opin Drug Del*, 14 (2017) 1-5.
- [6] J.W. Nichols, Y.H. Bae, EPR: Evidence and fallacy, *J Control Release*, 190 (2014) 451-464.
- [7] H.P. Gerber, P.D. Senter, I.S. Grewal, Antibody drug-conjugates targeting the tumor vasculature: Current and future developments, *MAbs*, 1 (2009) 247-253.
- [8] K.A. Kurdziel, J.D. Kalen, J.I. Hirsch, J.D. Wilson, H.D. Bear, J. Logan, J. McCumisky, K. Moorman-Sykes, S. Adler, P.L. Choyke, Human dosimetry and preliminary tumor distribution of 18F-fluoropaclitaxel in healthy volunteers and newly diagnosed breast cancer patients using PET/CT, *J Nucl Med*, 52 (2011) 1339-1345.
- [9] R.K. Jain, T. Stylianopoulos, Delivering nanomedicine to solid tumors, *Nat Rev Clin Oncol*, 7 (2010) 653-664.
- [10] D. Fukumura, R.K. Jain, Tumor microenvironment abnormalities: causes, consequences, and strategies to normalize, *J Cell Biochem*, 101 (2007) 937-949.
- [11] N.K. Reitan, M. Thuen, P.E. Goa, C.d.L. Davies, Characterization of tumor microvascular structure and permeability: comparison between magnetic resonance imaging and intravital confocal imaging, *J Biomed Opt*, 15 (2010) 036004.
- [12] C. He, C. Chan, R.R. Weichselbaum, G.F. Fleming, S.D. Yamada, W. Lin, Nanomedicine for Combination Therapy of Cancer, *EBioMedicine*, 2 (2015) 366-367.
- [13] H. Maeda, Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity, *Adv Drug Deliv Rev*, 91 (2015) 3-6.
- [14] U. Prabhakar, H. Maeda, R.K. Jain, E.M. Sevick-Muraca, W. Zamboni, O.C. Farokhzad, S.T. Barry, A. Gabizon, P. Grodzinski, D.C. Blakey, Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology, *Cancer Res*, 73 (2013) 2412-2417.
- [15] P.A. Netti, D.A. Berk, M.A. Swartz, A.J. Grodzinsky, R.K. Jain, Role of extracellular matrix assembly in interstitial transport in solid tumors, *Cancer Res*, 60 (2000) 2497-2503.

553 [16] L. Eikenes, O.S. Bruland, C. Brekken, L. Davies Cde, Collagenase increases the  
554 transcapillary pressure gradient and improves the uptake and distribution of  
555 monoclonal antibodies in human osteosarcoma xenografts, *Cancer Res*, 64 (2004) 4768-  
556 4773.

557 [17] L. Eikenes, M. Tari, I. Tufto, O.S. Bruland, C.D. Davies, Hyaluronidase induces a  
558 transcapillary pressure gradient and improves the distribution and uptake of liposomal  
559 doxorubicin (Caelyx (TM)) in human osteosarcoma xenografts, *Brit J Cancer*, 93 (2005)  
560 81-88.

561 [18] Y. Boucher, L.T. Baxter, R.K. Jain, Interstitial pressure gradients in tissue-isolated  
562 and subcutaneous tumors: implications for therapy, *Cancer Res*, 50 (1990) 4478-4484.

563 [19] V.P. Chauhan, T. Stylianopoulos, Y. Boucher, R.K. Jain, Delivery of molecular and  
564 nanoscale medicine to tumors: transport barriers and strategies, *Annu Rev Chem*  
565 *Biomol Eng*, 2 (2011) 281-298.

566 [20] Q. Dai, S. Wilhelm, D. Ding, A.M. Syed, S. Sindhvani, Y. Zhang, Y.Y. Chen, P.  
567 MacMillan, W.C.W. Chan, Quantifying the Ligand-Coated Nanoparticle Delivery to Cancer  
568 Cells in Solid Tumors, *ACS Nano*, 12 ( 2018 ) 8423-8435

569 \* An important work which exemplifies the delivery problem of nanomedicine

570 [21] S. Wilhelm, A.J. Tavares, Q. Dai, S. Ohta, J. Audet, H.F. Dvorak, W.C.W. Chan, Analysis  
571 of nanoparticle delivery to tumours, *Nat Rev Mater*, 1 (2016).

572 [22] N.J. Abbott, A.A. Patabendige, D.E. Dolman, S.R. Yusof, D.J. Begley, Structure and  
573 function of the blood-brain barrier, *Neurobiol Dis*, 37 (2010) 13-25.

574 [23] T. Ojha, V. Pathak, Y. Shi, W.E. Hennink, C.T.W. Moonen, G. Storm, F. Kiessling, T.  
575 Lammers, Pharmacological and physical vessel modulation strategies to improve EPR-  
576 mediated drug targeting to tumors, *Adv Drug Deliver Rev*, 119 (2017) 44-60.

577 [24] H. Kobayashi, R. Watanabe, P.L. Choyke, Improving conventional enhanced  
578 permeability and retention (EPR) effects; what is the appropriate target?, *Theranostics*,  
579 4 (2013) 81-89.

580 [25] S. Mura, J. Nicolas, P. Couvreur, Stimuli-responsive nanocarriers for drug delivery,  
581 *Nat Mater*, 12 (2013) 991-1003.

582 [26] F. Danhier, To exploit the tumor microenvironment: Since the EPR effect fails in the  
583 clinic, what is the future of nanomedicine?, *J Control Release*, 244 (2016) 108-121.

584 [27] Y.H. Bae, K. Park, Targeted drug delivery to tumors: myths, reality and possibility, *J*  
585 *Control Release*, 153 (2011) 198-205.

586 [28] A. Rix, W. Lederle, B. Theek, T. Lammers, C. Moonen, G. Schmitz, F. Kiessling,  
587 Advanced Ultrasound Technologies for Diagnosis and Therapy, *J Nucl Med*, (2018).

588 [29] C.A. Sennoga, E. Kanbar, L. Auboire, P.A. Dujardin, D. Fouan, J.M. Escoffre, A.  
589 Bouakaz, Microbubble-mediated ultrasound drug-delivery and therapeutic monitoring,  
590 *Expert Opin Drug Del*, 14 (2017) 1031-1043.

591 [30] L. Lamsam, E. Johnson, I.D. Connolly, M. Wintermark, M. Hayden Gephart, A review  
592 of potential applications of MR-guided focused ultrasound for targeting brain tumor  
593 therapy, *Neurosurg Focus*, 44 (2018) E10.

594 [31] G.A. Hussein, W.G. Pitt, A.M. Martins, Ultrasonically triggered drug delivery:  
595 breaking the barrier, *Colloids Surf B Biointerfaces*, 123 (2014) 364-386.

596 [32] V. Frenkel, Ultrasound mediated delivery of drugs and genes to solid tumors, *Adv*  
597 *Drug Deliv Rev*, 60 (2008) 1193-1208.

598 [33] W.D. O'Brien, Jr., Ultrasound-biophysics mechanisms, *Prog Biophys Mol Biol*, 93  
599 (2007) 212-255.

600 [34] W.G. Pitt, G.A. Hussein, B.J. Staples, Ultrasonic drug delivery--a general review,  
601 *Expert Opin Drug Deliv*, 1 (2004) 37-56.



602 [35] D.O. Draper, J.C. Castel, D. Castel, Rate of temperature increase in human muscle  
603 during 1 MHz and 3 MHz continuous ultrasound, *J Orthop Sports Phys Ther*, 22 (1995)  
604 142-150.

605 [36] J.E. Kennedy, G.R. Ter Haar, D. Cranston, High intensity focused ultrasound: surgery  
606 of the future?, *Br J Radiol*, 76 (2003) 590-599.

607 [37] P.E. Huber, J.W. Jenne, R. Rastert, I. Simiantonakis, H.P. Sinn, H.J. Strittmatter, D. von  
608 Fournier, M.F. Wannemacher, J. Debus, A new noninvasive approach in breast cancer  
609 therapy using magnetic resonance imaging-guided focused ultrasound surgery, *Cancer*  
610 *Res*, 61 (2001) 8441-8447.

611 [38] H. Grull, S. Langereis, Hyperthermia-triggered drug delivery from temperature-  
612 sensitive liposomes using MRI-guided high intensity focused ultrasound, *J Control*  
613 *Release*, 161 (2012) 317-327.

614 [39] G. Kong, M.W. Dewhirst, Hyperthermia and liposomes, *Int J Hyperthermia*, 15  
615 (1999) 345-370.

616 [40] O. Couture, J. Foley, N.F. Kassell, B. Larrat, J.-F. Aubry, Review of ultrasound  
617 mediated drug delivery for cancer treatment: updates from preclinical studies, *Transl*  
618 *Cancer Res* 3(2014) 494-511.

619 [41] C.W. Song, Effect of local hyperthermia on blood flow and microenvironment: a  
620 review, *Cancer Res*, 44 (1984) 4721s-4730s.

621 [42] G. Kong, G. Anyarambhatla, W.P. Petros, R.D. Braun, O.M. Colvin, D. Needham, M.W.  
622 Dewhirst, Efficacy of liposomes and hyperthermia in a human tumor xenograft model:  
623 importance of triggered drug release, *Cancer Res*, 60 (2000) 6950-6957.

624 [43] A. Yudina, C. Moonen, Ultrasound-induced cell permeabilisation and hyperthermia:  
625 strategies for local delivery of compounds with intracellular mode of action, *Int J*  
626 *Hyperthermia*, 28 (2012) 311-319.

627 [44] A. Jain, A. Tiwari, A. Verma, S.K. Jain, Ultrasound-based triggered drug delivery to  
628 tumors, *Drug Deliv Transl Res*, 8 (2018) 150-164.

629 [45] P. Dayton, A. Klibanov, G. Brandenburger, K. Ferrara, Acoustic radiation force in  
630 vivo: a mechanism to assist targeting of microbubbles, *Ultrasound Med Biol*, 25 (1999)  
631 1195-1201.

632 [46] S.O. Dymling, H.W. Persson, T.G. Hertz, K. Lindstrom, A New Ultrasonic Method for  
633 Fluid Property Measurements, *Ultrasound in Medicine and Biology*, 17 (1991) 497-500.

634 [47] G. Dimcevski, S. Kotopoulis, T. Bjanes, D. Hoem, J. Schjott, B.T. Gjertsen, M.  
635 Biermann, A. Molven, H. Sorbye, E. McCormack, M. Postema, O.H. Gilja, A human clinical  
636 trial using ultrasound and microbubbles to enhance gemcitabine treatment of  
637 inoperable pancreatic cancer, *J Control Release*, 243 (2016) 172-181.

638 \*\* First clinical trial demonstrating sonopermeation in patients with pancreatic cancer  
639 [48] S. Kotopoulis, G. Dimcevski, O.H. Gilja, D. Hoem, M. Postema, Treatment of human  
640 pancreatic cancer using combined ultrasound, microbubbles, and gemcitabine: A clinical  
641 case study, *Med Phys*, 40 (2013).

642 [49] A. Carpentier, M. Canney, A. Vignot, V. Reina, K. Beccaria, C. Horodyckid, C. Karachi,  
643 D. Leclercq, C. Lafon, J.Y. Chapelon, L. Capelle, P. Cornu, M. Sanson, K. Hoang-Xuan, J.Y.  
644 Delattre, A. Idbaih, Clinical trial of blood-brain barrier disruption by pulsed ultrasound,  
645 *Sci Transl Med*, 8 (2016) 343.

646 \*\* Describes sonopermeation in a clinical study on glioblastoma  
647 [50] G. Lajoinie, I. De Cock, C.C. Coussios, I. Lentacker, S. Le Gac, E. Stride, M. Versluis, In  
648 vitro methods to study bubble-cell interactions: Fundamentals and therapeutic  
649 applications, *Biomicrofluidics*, 10 (2016) 011501.



650 [51] I. Lentacker, I. De Cock, R. Deckers, S.C. De Smedt, C.T. Moonen, Understanding  
651 ultrasound induced sonoporation: definitions and underlying mechanisms, *Adv Drug*  
652 *Deliv Rev*, 72 (2014) 49-64.

653 \* Review paper which describes the mechanisms of sonopermeation

654 [52] B.H.A. Lammertink, C. Bos, R. Deckers, G. Storm, C.T.W. Moonen, J.M. Escoffre,  
655 Sonochemotherapy: from bench to bedside, *Front Pharmacol*, 6 (2015).

656 \*\* A nice review summarizing sonopermeation to tumors

657 [53] T. Boissenot, A. Bordat, E. Fattal, N. Tsapis, Ultrasound-triggered drug delivery for  
658 cancer treatment using drug delivery systems: From theoretical considerations to  
659 practical applications, *J Control Release*, 241 (2016) 144-163.

660 [54] H.L. Liu, C.H. Fan, C.Y. Ting, C.K. Yeh, Combining microbubbles and ultrasound for  
661 drug delivery to brain tumors: current progress and overview, *Theranostics*, 4 (2014)  
662 432-444.

663 [55] J.P. Ross, X. Cai, J.F. Chiu, J. Yang, J. Wu, Optical and atomic force microscopic studies  
664 on sonoporation, *J Acoust Soc Am*, 111 (2002) 1161-1164.

665 [56] S. Bao, B.D. Thrall, D.L. Miller, Transfection of a reporter plasmid into cultured cells  
666 by sonoporation in vitro, *Ultrasound Med Biol*, 23 (1997) 953-959.

667 [57] N. Sheikov, N. McDannold, S. Sharma, K. Hynynen, Effect of focused ultrasound  
668 applied with an ultrasound contrast agent on the tight junctional integrity of the brain  
669 microvascular endothelium, *Ultrasound Med Biol*, 34 (2008) 1093-1104.

670 [58] B.D. Meijering, L.J. Juffermans, A. van Wamel, R.H. Henning, I.S. Zuhorn, M. Emmer,  
671 A.M. Versteilen, W.J. Paulus, W.H. van Gilst, K. Kooiman, N. de Jong, R.J. Musters, L.E.  
672 Deelman, O. Kamp, Ultrasound and microbubble-targeted delivery of macromolecules is  
673 regulated by induction of endocytosis and pore formation, *Circ Res*, 104 (2009) 679-  
674 687.

675 [59] Y. Yuana, L. Jiang, B.H.A. Lammertink, P. Vader, R. Deckers, C. Bos, R.M. Schiffelers,  
676 C.T. Moonen, Microbubbles-Assisted Ultrasound Triggers the Release of Extracellular  
677 Vesicles, *Int J Mol Sci*, 18 (2017).

678 [60] A. Rix, M. Palmowski, F. Gremse, K. Palmowski, W. Lederle, F. Kiessling, J. Bzyl,  
679 Influence of Repetitive Contrast Agent Injections on Functional and Molecular  
680 Ultrasound Measurements, *Ultrasound in Medicine and Biology*, 40 (2014) 2468-2475.

681 [61] D.S. Hersh, B.A. Nguyen, J.G. Dancy, A.R. Adapa, J.A. Winkles, G.F. Woodworth, A.J.  
682 Kim, V. Frenkel, Pulsed ultrasound expands the extracellular and perivascular spaces of  
683 the brain, *Brain Res*, 1646 (2016) 543-550.

684 [62] J. Collis, R. Manasseh, P. Liovic, P. Tho, A. Ooi, K. Petkovic-Duran, Y. Zhu, Cavitation  
685 microstreaming and stress fields created by microbubbles, *Ultrasonics*, 50 (2010) 273-  
686 279.

687 [63] S.A. Elder, Cavitation microstreaming, *J Acoust Soc Am*, 31 (1959) 54-64.

688 [64] K. Kooiman, H.J. Vos, M. Versluis, N. de Jong, Acoustic behavior of microbubbles and  
689 implications for drug delivery, *Adv Drug Deliv Rev*, 72 (2014) 28-48.

690 [65] A. van Wamel, K. Kooiman, M. Hartevelde, M. Emmer, F.J. ten Cate, M. Versluis, N. de  
691 Jong, Vibrating microbubbles poking individual cells: drug transfer into cells via  
692 sonoporation, *J Control Release*, 112 (2006) 149-155.

693 [66] C. Poon, D. McMahon, K. Hynynen, Noninvasive and targeted delivery of  
694 therapeutics to the brain using focused ultrasound, *Neuropharmacology*, 120 (2017)  
695 20-37.

696 \* Review paper which describes sonopermeation in the brain

697 [67] A. Schroeder, J. Kost, Y. Barenholz, Ultrasound, liposomes, and drug delivery:  
698 principles for using ultrasound to control the release of drugs from liposomes, *Chem*  
699 *Phys Lipids*, 162 (2009) 1-16.

700 [68] K. Hynynen, N. McDannold, N. Vykhodtseva, F.A. Jolesz, Noninvasive MR imaging-  
701 guided focal opening of the blood-brain barrier in rabbits, *Radiology*, 220 (2001) 640-  
702 646.

703 [69] K. Hynynen, N. McDannold, N.A. Sheikov, F.A. Jolesz, N. Vykhodtseva, Local and  
704 reversible blood-brain barrier disruption by noninvasive focused ultrasound at  
705 frequencies suitable for trans-skull sonications, *Neuroimage*, 24 (2005) 12-20.

706 [70] N. McDannold, N. Vykhodtseva, K. Hynynen, Blood-brain barrier disruption induced  
707 by focused ultrasound and circulating preformed microbubbles appears to be  
708 characterized by the mechanical index, *Ultrasound Med Biol*, 34 (2008) 834-840.

709 [71] N. McDannold, N. Vykhodtseva, K. Hynynen, Effects of acoustic parameters and  
710 ultrasound contrast agent dose on focused-ultrasound induced blood-brain barrier  
711 disruption, *Ultrasound Med Biol*, 34 (2008) 930-937.

712 [72] G. Samiotaki, E.E. Konofagou, Dependence of the reversibility of focused-  
713 ultrasound-induced blood-brain barrier opening on pressure and pulse length in vivo,  
714 *IEEE Trans Ultrason Ferroelectr Freq Control*, 60 (2013) 2257-2265.

715 [73] A.N. Pouliopoulos, C. Li, M. Tinguely, V. Garbin, M.X. Tang, J.J. Choi, Rapid short-  
716 pulse sequences enhance the spatiotemporal uniformity of acoustically driven  
717 microbubble activity during flow conditions, *J Acoust Soc Am*, 140 (2016) 2469.

718 [74] A. Dasgupta, M. Liu, T. Ojha, G. Storm, F. Kiessling, T. Lammers, Ultrasound-  
719 mediated drug delivery to the brain: principles, progress and prospects, *Drug Discov*  
720 *Today Technol*, 20 (2016) 41-48.

721 [75] A. Burgess, S. Dubey, S. Yeung, O. Hough, N. Eterman, I. Aubert, K. Hynynen,  
722 Alzheimer disease in a mouse model: MR imaging-guided focused ultrasound targeted  
723 to the hippocampus opens the blood-brain barrier and improves pathologic  
724 abnormalities and behavior, *Radiology*, 273 (2014) 736-745.

725 [76] M.A. O'Reilly, K. Hynynen, Blood-brain barrier: real-time feedback-controlled  
726 focused ultrasound disruption by using an acoustic emissions-based controller,  
727 *Radiology*, 263 (2012) 96-106.

728 [77] V.A. Salgaonkar, S. Datta, C.K. Holland, T.D. Mast, Passive cavitation imaging with  
729 ultrasound arrays, *J Acoust Soc Am*, 126 (2009) 3071-3083.

730 [78] E.A. Neppiras, Acoustic cavitation, *Physics Reports*, 61 (1980) 159-251.

731 [79] S.K. Wu, P.C. Chu, W.Y. Chai, S.T. Kang, C.H. Tsai, C.H. Fan, C.K. Yeh, H.L. Liu,  
732 Characterization of Different Microbubbles in Assisting Focused Ultrasound-Induced  
733 Blood-Brain Barrier Opening, *Sci Rep*, 7 (2017) 46689.

734 [80] N. McDannold, N. Vykhodtseva, K. Hynynen, Targeted disruption of the blood-brain  
735 barrier with focused ultrasound: association with cavitation activity, *Phys Med Biol*, 51  
736 (2006) 793-807.

737 [81] Y.S. Tung, F. Vlachos, J.J. Choi, T. Deffieux, K. Selert, E.E. Konofagou, In vivo  
738 transcranial cavitation threshold detection during ultrasound-induced blood-brain  
739 barrier opening in mice, *Phys Med Biol*, 55 (2010) 6141-6155.

740 [82] A. Delalande, S. Kotopoulos, M. Postema, P. Midoux, C. Pichon, Sonoporation:  
741 mechanistic insights and ongoing challenges for gene transfer, *Gene*, 525 (2013) 191-  
742 199.

743 [83] M. Afadzi, S.P. Strand, E.A. Nilssen, S.E. Masoy, T.F. Johansen, R. Hansen, B.A.  
744 Angelsen, L.D.C. de, Mechanisms of the ultrasound-mediated intracellular delivery of  
745 liposomes and dextrans, *IEEE Trans Ultrason Ferroelectr Freq Control*, 60 (2013) 21-33.

746 [84] H. Dewitte, S. Van Lint, C. Heirman, K. Thielemans, S.C. De Smedt, K. Breckpot, I.  
747 Lentacker, The potential of antigen and TriMix sonoporation using mRNA-loaded  
748 microbubbles for ultrasound-triggered cancer immunotherapy, *J Control Release*, 194  
749 (2014) 28-36.

750 [85] R. Karshafian, S. Samac, P.D. Bevan, P.N. Burns, Microbubble mediated  
751 sonoporation of cells in suspension: clonogenic viability and influence of molecular size  
752 on uptake, *Ultrasonics*, 50 (2010) 691-697.

753 [86] Z. Fan, H. Liu, M. Mayer, C.X. Deng, Spatiotemporally controlled single cell  
754 sonoporation, *Proc Natl Acad Sci U S A*, 109 (2012) 16486-16491.

755 [87] Y.Z. Zhao, Y.K. Luo, C.T. Lu, J.F. Xu, J. Tang, M. Zhang, Y. Zhang, H.D. Liang,  
756 Phospholipids-based microbubbles sonoporation pore size and reseal of cell membrane  
757 cultured in vitro, *J Drug Target*, 16 (2008) 18-25.

758 [88] Y. Hu, J.M. Wan, A.C. Yu, Membrane perforation and recovery dynamics in  
759 microbubble-mediated sonoporation, *Ultrasound Med Biol*, 39 (2013) 2393-2405.

760 [89] A. Yudina, M. Lepetit-Coiffe, C.T. Moonen, Evaluation of the temporal window for  
761 drug delivery following ultrasound-mediated membrane permeability enhancement,  
762 *Mol Imaging Biol*, 13 (2011) 239-249.

763 [90] I. Skachkov, Y. Luan, A. van der Steen, N. de Jong, K. Kooiman, Targeted  
764 microbubble mediated sonoporation of endothelial cells in vivo, *Ultrasonics*,  
765 *Ferroelectrics, and Frequency Control*, IEEE Transactions on, 61 (2014) 1661-1667.

766 [91] A. van Wamel, P.C. Sontum, A. Healey, S. Kvale, N. Bush, J. Bamber, C. de Lange  
767 Davies, Acoustic Cluster Therapy (ACT) enhances the therapeutic efficacy of paclitaxel  
768 and Abraxane(R) for treatment of human prostate adenocarcinoma in mice, *J Control*  
769 *Release*, 236 (2016) 15-21.

770 \*\* Describes a custom made microbubble system for drug delivery

771 [92] C.W. Burke, E.t. Alexander, K. Timbie, A.L. Kilbanov, R.J. Price, Ultrasound-activated  
772 agents comprised of 5FU-bearing nanoparticles bonded to microbubbles inhibit solid  
773 tumor growth and improve survival, *Mol Ther*, 22 (2014) 321-328.

774 [93] S. Kotopoulos, A. Delalande, M. Popa, V. Mamaeva, G. Dimcevski, O.H. Gilja, M.  
775 Postema, B.T. Gjertsen, E. McCormack, Sonoporation-enhanced chemotherapy  
776 significantly reduces primary tumour burden in an orthotopic pancreatic cancer  
777 xenograft, *Mol Imaging Biol*, 16 (2014) 53-62.

778 [94] C.Y. Lin, Y.L. Huang, J.R. Li, F.H. Chang, W.L. Lin, Effects of focused ultrasound and  
779 microbubbles on the vascular permeability of nanoparticles delivered into mouse  
780 tumors, *Ultrasound Med Biol*, 36 (2010) 1460-1469.

781 [95] C.Y. Lin, J.R. Li, H.C. Tseng, M.F. Wu, W.L. Lin, Enhancement of focused ultrasound  
782 with microbubbles on the treatments of anticancer nanodrug in mouse tumors,  
783 *Nanomedicine*, 8 (2012) 900-907.

784 [96] T.Y. Wang, J.W. Choe, K. Pu, R. Devulapally, S. Bachawal, S. Machtaler, S.M.  
785 Chowdhury, R. Luong, L. Tian, B. Khuri-Yakub, J. Rao, R. Paulmurugan, J.K. Willmann,  
786 Ultrasound-guided delivery of microRNA loaded nanoparticles into cancer, *J Control*  
787 *Release*, 203 (2015) 99-108.

788 [97] C.Y. Lin, T.M. Liu, C.Y. Chen, Y.L. Huang, W.K. Huang, C.K. Sun, F.H. Chang, W.L. Lin,  
789 Quantitative and qualitative investigation into the impact of focused ultrasound with  
790 microbubbles on the triggered release of nanoparticles from vasculature in mouse  
791 tumors, *J Control Release*, 146 (2010) 291-298.

792 [98] D.S. Hersh, A.S. Wadajkar, N. Roberts, J.G. Perez, N.P. Connolly, V. Frenkel, J.A.  
793 Winkles, G.F. Woodworth, A.J. Kim, Evolving Drug Delivery Strategies to Overcome the  
794 Blood Brain Barrier, *Curr Pharm Des*, 22 (2016) 1177-1193.

795 [99] N. Sheikov, N. McDannold, N. Vykhodtseva, F. Jolesz, K. Hynynen, Cellular  
796 mechanisms of the blood-brain barrier opening induced by ultrasound in presence of  
797 microbubbles, *Ultrasound Med Biol*, 30 (2004) 979-989.

798 [100] B. Zhao, Y. Chen, J. Liu, L. Zhang, J. Wang, Y. Yang, Q. Lv, M. Xie, Blood-brain barrier  
799 disruption induced by diagnostic ultrasound combined with microbubbles in mice,  
800 *Oncotarget*, 9 (2018) 4897-4914.

801 [101] N. Sheikov, N. McDannold, F. Jolesz, Y.Z. Zhang, K. Tam, K. Hynynen, Brain  
802 arterioles show more active vesicular transport of blood-borne tracer molecules than  
803 capillaries and venules after focused ultrasound-evoked opening of the blood-brain  
804 barrier, *Ultrasound Med Biol*, 32 (2006) 1399-1409.

805 [102] J. Deng, Q. Huang, F. Wang, Y. Liu, Z. Wang, Z. Wang, Q. Zhang, B. Lei, Y. Cheng, The  
806 role of caveolin-1 in blood-brain barrier disruption induced by focused ultrasound  
807 combined with microbubbles, *J Mol Neurosci*, 46 (2012) 677-687.

808 [103] M. Aryal, K. Fischer, C. Gentile, S. Gitto, Y.Z. Zhang, N. McDannold, Effects on P-  
809 Glycoprotein Expression after Blood-Brain Barrier Disruption Using Focused  
810 Ultrasound and Microbubbles, *Plos One*, 12 (2017) e0166061.

811 [104] H. Cho, H.Y. Lee, M. Han, J.R. Choi, S. Ahn, T. Lee, Y. Chang, J. Park, Localized Down-  
812 regulation of P-glycoprotein by Focused Ultrasound and Microbubbles induced Blood-  
813 Brain Barrier Disruption in Rat Brain, *Sci Rep*, 6 (2016) 31201.

814 [105] P. Hadaczek, Y. Yamashita, H. Mirek, L. Tamas, M.C. Bohn, C. Noble, J.W. Park, K.  
815 Bankiewicz, The "perivascular pump" driven by arterial pulsation is a powerful  
816 mechanism for the distribution of therapeutic molecules within the brain, *Mol Ther*, 14  
817 (2006) 69-78.

818 [106] H. Chen, G.Z. Yang, H. Getachew, C. Acosta, C. Sierra Sanchez, E.E. Konofagou,  
819 Focused ultrasound-enhanced intranasal brain delivery of brain-derived neurotrophic  
820 factor, *Sci Rep*, 6 (2016) 28599.

821 [107] S.B. Raymond, J. Skoch, K. Hynynen, B.J. Bacskai, Multiphoton imaging of  
822 ultrasound/Optison mediated cerebrovascular effects in vivo, *J Cereb Blood Flow Metab*,  
823 27 (2007) 393-403.

824 [108] D.E. Goertz, M. Todorova, O. Mortazavi, V. Agache, B. Chen, R. Karshafian, K.  
825 Hynynen, Antitumor effects of combining docetaxel (taxotere) with the antivasular  
826 action of ultrasound stimulated microbubbles, *Plos One*, 7 (2012) e52307.

827 [109] C.P. Keravnou, I. De Cock, I. Lentacker, M.L. Izamis, M.A. Averkiou, Microvascular  
828 Injury and Perfusion Changes Induced by Ultrasound and Microbubbles in a Machine-  
829 Perfused Pig Liver, *Ultrasound in Medicine and Biology*, 42 (2016) 2676-2686.

830 [110] X. Hu, A. Kheirrolomoom, L.M. Mahakian, J.R. Beegle, D.E. Kruse, K.S. Lam, K.W.  
831 Ferrara, Insonation of targeted microbubbles produces regions of reduced blood flow  
832 within tumor vasculature, *Invest Radiol*, 47 (2012) 398-405.

833 [111] A. El Kaffas, M.J. Gangeh, G. Farhat, W.T. Tran, A. Hashim, A. Giles, G.J. Czarnota,  
834 Tumour Vascular Shutdown and Cell Death Following Ultrasound-Microbubble  
835 Enhanced Radiation Therapy, *Theranostics*, 8 (2018) 314-327.

836 [112] J.T. Belcik, B.H. Mott, A. Xie, Y. Zhao, S. Kim, N.J. Lindner, A. Ammi, J.M. Linden, J.R.  
837 Lindner, Augmentation of limb perfusion and reversal of tissue ischemia produced by  
838 ultrasound-mediated microbubble cavitation, *Circ Cardiovasc Imaging*, 8 (2015).

839 [113] V. Paefgen, D. Doleschel, F. Kiessling, Evolution of contrast agents for ultrasound  
840 imaging and ultrasound-mediated drug delivery, *Front Pharmacol*, 6 (2015) 197.

841 [114] R. Suzuki, A.L. Klibanov, Co-administration of Microbubbles and Drugs in  
842 Ultrasound-Assisted Drug Delivery: Comparison with Drug-Carrying Particles, *Adv Exp*  
843 *Med Biol*, 880 (2016) 205-220.

844 [115] I. Lentacker, S.C.D. Smedt, N.N. Sanders, Drug loaded microbubble design for  
845 ultrasound triggered delivery, *Soft Matter*, 5 (2009) 2161–2170.

846 [116] F. Kiessling, S. Fokong, P. Koczera, W. Lederle, T. Lammers, Ultrasound  
847 microbubbles for molecular diagnosis, therapy, and theranostics, *J Nucl Med*, 53 (2012)  
848 345-348.

849 [117] B. Theek, M. Baues, T. Ojha, D. Mockel, S.K. Veettil, J. Steitz, L. van Bloois, G. Storm,  
850 F. Kiessling, T. Lammers, Sonoporation enhances liposome accumulation and  
851 penetration in tumors with low EPR, *J Control Release*, 231 (2016) 77-85.

852 [118] M.L. De Temmerman, H. Dewitte, R.E. Vandenbroucke, B. Lucas, C. Libert, J.  
853 Demeester, S.C. De Smedt, I. Lentacker, J. Rejman, mRNA-Lipoplex loaded microbubble  
854 contrast agents for ultrasound-assisted transfection of dendritic cells, *Biomaterials*, 32  
855 (2011) 9128-9135.

856 [119] C.W. Burke, Y.H.J. Hsiang, E. Alexander, A.L. Kilbanov, R.J. Price, Covalently Linking  
857 Poly(lactic-co-glycolic acid) Nanoparticles to Microbubbles Before Intravenous Injection  
858 Improves their Ultrasound-Targeted Delivery to Skeletal Muscle, *Small*, 7 (2011) 1227-  
859 1235.

860 [120] I. De Cock, G. Lajoinie, M. Versluis, S.C. De Smedt, I. Lentacker, Sonoprinting and  
861 the importance of microbubble loading for the ultrasound mediated cellular delivery of  
862 nanoparticles, *Biomaterials*, 83 (2016) 294-307.

863 [121] S. Snipstad, S. Berg, Y. Morch, A. Bjorkoy, E. Sulheim, R. Hansen, I. Grimstad, A. van  
864 Wamel, A.F. Maaland, S.H. Torp, C.L. Davies, Ultrasound Improves the Delivery and  
865 Therapeutic Effect of Nanoparticle-Stabilized Microbubbles in Breast Cancer Xenografts,  
866 *Ultrasound Med Biol*, 43 (2017) 2651-2669.

867 [122] P. Koczera, L. Appold, Y. Shi, M. Liu, A. Dasgupta, V. Pathak, T. Ojha, S. Fokong, Z.  
868 Wu, M. van Zandvoort, O. Iranzo, A.J. Kuehne, A. Pich, F. Kiessling, T. Lammers, PBCA-  
869 based polymeric microbubbles for molecular imaging and drug delivery, *J Control*  
870 *Release*, (2017).

871 [123] N. Rapoport, Drug-Loaded Perfluorocarbon Nanodroplets for Ultrasound-  
872 Mediated Drug Delivery, *Therapeutic Ultrasound*, 880 (2016) 221-241.

873 [124] J.E. Silpe, J.K. Nunes, A.T. Poortinga, H.A. Stone, Generation of antibubbles from  
874 core-shell double emulsion templates produced by microfluidics, *Langmuir*, 29 (2013)  
875 8782-8787.

876 [125] A. Hughes, K. Hynynen, Design of patient-specific focused ultrasound arrays for  
877 non-invasive brain therapy with increased trans-skull transmission and steering range,  
878 *Physics in Medicine and Biology*, 62 (2017) L9-L19.

879 [126] Z.G. Li, A.Q. Liu, E. Klaseboer, J.B. Zhang, C.D. Ohl, Single cell membrane poration  
880 by bubble-induced microjets in a microfluidic chip, *Lab Chip*, 13 (2013) 1144-1150.

881 [127] B. Helfield, X. Chen, S.C. Watkins, F.S. Villanueva, Biophysical insight into  
882 mechanisms of sonoporation, *Proc Natl Acad Sci U S A*, 113 (2016) 9983-9988.

883 \*\* A detailed description of in vitro pore formation

884 [128] I. De Cock, E. Zagato, K. Braeckmans, Y. Luan, N. de Jong, S.C. De Smedt, I.  
885 Lentacker, Ultrasound and microbubble mediated drug delivery: acoustic pressure as  
886 determinant for uptake via membrane pores or endocytosis, *J Control Release*, 197  
887 (2015) 20-28.

888 [129] S.J. Grainger, J.V. Serna, S. Sunny, Y. Zhou, C.X. Deng, M.E. El-Sayed, Pulsed  
889 ultrasound enhances nanoparticle penetration into breast cancer spheroids, *Mol Pharm*,  
890 7 (2010) 2006-2019.

891 [130] Y.C. Park, C. Zhang, S. Kim, G. Mohamedi, C. Beigie, J.O. Nagy, R.G. Holt, R.O.  
892 Cleveland, N.L. Jeon, J.Y. Wong, Microvessels-on-a-Chip to Assess Targeted Ultrasound-  
893 Assisted Drug Delivery, *ACS Appl Mater Interfaces*, 8 (2016) 31541-31549.

894 [131] A. Herland, A.D. van der Meer, E.A. FitzGerald, T.E. Park, J.J. Sleeboom, D.E. Ingber,  
895 Distinct Contributions of Astrocytes and Pericytes to Neuroinflammation Identified in a  
896 3D Human Blood-Brain Barrier on a Chip, *Plos One*, 11 (2016) e0150360.

897 [132] B.M. Maoz, A. Herland, E.A. FitzGerald, T. Grevesse, C. Vidoudez, A.R. Pacheco, S.P.  
898 Sheehy, T.E. Park, S. Dauth, R. Mannix, N. Budnik, K. Shores, A. Cho, J.C. Nawroth, D.  
899 Segre, B. Budnik, D.E. Ingber, K.K. Parker, A linked organ-on-chip model of the human  
900 neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells, *Nat*  
901 *Biotechnol*, (2018).

902 [133] H. Chen, A.A. Brayman, T.J. Matula, Characteristic microvessel relaxation  
903 timescales associated with ultrasound-activated microbubbles, *Appl Phys Lett*, 101  
904 (2012) 163704.

905 [134] N. Hosseinkhah, H. Chen, T.J. Matula, P.N. Burns, K. Hynynen, Mechanisms of  
906 microbubble-vessel interactions and induced stresses: a numerical study, *J Acoust Soc*  
907 *Am*, 134 (2013) 1875-1885.

908 [135] A. van Wamel, A. Bouakaz, M. Versluis, N. de Jong, Micromanipulation of  
909 endothelial cells: ultrasound-microbubble-cell interaction, *Ultrasound Med Biol*, 30  
910 (2004) 1255-1258.

911 [136] N. Kudo, K. Okada, K. Yamamoto, Sonoporation by single-shot pulsed ultrasound  
912 with microbubbles adjacent to cells, *Biophys J*, 96 (2009) 4866-4876.

913 [137] P. Prentice, A. Cuschieri, K. Dholakia, M. Prausnitz, P. Campbell, Membrane  
914 disruption by optically controlled microbubble cavitation, *Nature Physics*, 1 (2005)  
915 107-110.

916 [138] S. Mehier-Humbert, T. Bettinger, F. Yan, R.H. Guy, Plasma membrane poration  
917 induced by ultrasound exposure: implication for drug delivery, *J Control Release*, 104  
918 (2005) 213-222.

919 [139] Y. Zhou, K. Yang, J. Cui, J.Y. Ye, C.X. Deng, Controlled permeation of cell membrane  
920 by single bubble acoustic cavitation, *J Control Release*, 157 (2012) 103-111.

921 [140] L. Fan, Y. Liu, H. Ying, Y. Xue, Z. Zhang, P. Wang, L. Liu, H. Zhang, Increasing of  
922 blood-tumor barrier permeability through paracellular pathway by low-frequency  
923 ultrasound irradiation in vitro, *J Mol Neurosci*, 43 (2011) 541-548.

924 [141] K. Kooiman, M. Foppen-Harteveld, A.F. van der Steen, N. de Jong, Sonoporation of  
925 endothelial cells by vibrating targeted microbubbles, *J Control Release*, 154 (2011) 35-  
926 41.

927 [142] H. Dewitte, K. Vanderperren, H. Haers, E. Stock, L. Duchateau, M. Hesta, J.H.  
928 Saunders, S.C. De Smedt, I. Lentacker, Theranostic mRNA-loaded Microbubbles in the  
929 Lymphatics of Dogs: Implications for Drug Delivery, *Theranostics*, 5 (2015) 97-109.

930 [143] K.-H. Song, B.K. Harvey, M.A. Borden, State-of-the-art of microbubble-assisted  
931 blood-brain barrier disruption *Theranostics*, 8 (2018) 4393-4408.

932 [144] J.M. Escoffre, R. Deckers, C. Bos, C. Moonen, Bubble-Assisted Ultrasound:  
933 Application in Immunotherapy and Vaccination, *Adv Exp Med Biol*, 880 (2016) 243-261.

934 [145] A. Burgess, K. Hynynen, Microbubble-Assisted Ultrasound for Drug Delivery in the  
935 Brain and Central Nervous System, *Adv Exp Med Biol*, 880 (2016) 293-308.

936 [146] J. Park, M. Aryal, N. Vykhodtseva, Y.Z. Zhang, N. McDannold, Evaluation of  
937 permeability, doxorubicin delivery, and drug retention in a rat brain tumor model after  
938 ultrasound-induced blood-tumor barrier disruption, *J Control Release*, 250 (2017) 77-  
939 85.

940 [147] A.K.O. Aslund, S. Berg, S. Hak, Y. Morch, S.H. Torp, A. Sandvig, M. Wideroe, R.  
941 Hansen, C. de Lange Davies, Nanoparticle delivery to the brain--By focused ultrasound  
942 and self-assembled nanoparticle-stabilized microbubbles, *J Control Release*, 220 (2015)  
943 287-294.

944 [148] L.H. Treat, N. McDannold, Y. Zhang, N. Vykhodtseva, K. Hynynen, Improved anti-  
945 tumor effect of liposomal doxorubicin after targeted blood-brain barrier disruption by  
946 MRI-guided focused ultrasound in rat glioma, *Ultrasound Med Biol*, 38 (2012) 1716-  
947 1725.

948 [149] M. Aryal, N. Vykhodtseva, Y.Z. Zhang, J. Park, N. McDannold, Multiple treatments  
949 with liposomal doxorubicin and ultrasound-induced disruption of blood-tumor and  
950 blood-brain barriers improve outcomes in a rat glioma model, *J Control Release*, 169  
951 (2013) 103-111.

952 [150] E.J. Park, Y.Z. Zhang, N. Vykhodtseva, N. McDannold, Ultrasound-mediated blood-  
953 brain/blood-tumor barrier disruption improves outcomes with trastuzumab in a breast  
954 cancer brain metastasis model, *J Control Release*, 163 (2012) 277-284.

955 [151] T. Kobus, I.K. Zervantonakis, Y. Zhang, N.J. McDannold, Growth inhibition in a  
956 brain metastasis model by antibody delivery using focused ultrasound-mediated blood-  
957 brain barrier disruption, *J Control Release*, 238 (2016) 281-288.

958 [152] M. Kinoshita, N. McDannold, F.A. Jolesz, K. Hynynen, Noninvasive localized  
959 delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced  
960 blood-brain barrier disruption, *Proc Natl Acad Sci U S A*, 103 (2006) 11719-11723.

961 [153] P.Y. Chen, H.Y. Hsieh, C.Y. Huang, C.Y. Lin, K.C. Wei, H.L. Liu, Focused ultrasound-  
962 induced blood-brain barrier opening to enhance interleukin-12 delivery for brain tumor  
963 immunotherapy: a preclinical feasibility study, *J Transl Med*, 13 (2015) 93.

964 [154] R. Alkins, A. Burgess, M. Ganguly, G. Francia, R. Kerbel, W.S. Wels, K. Hynynen,  
965 Focused ultrasound delivers targeted immune cells to metastatic brain tumors, *Cancer*  
966 *Res*, 73 (2013) 1892-1899.

967 [155] R. Alkins, A. Burgess, R. Kerbel, W.S. Wels, K. Hynynen, Early treatment of HER2-  
968 amplified brain tumors with targeted NK-92 cells and focused ultrasound improves  
969 survival, *Neuro Oncol*, 18 (2016) 974-981.

970 [156] F. Marquet, Y.S. Tung, T. Teichert, V.P. Ferrera, E.E. Konofagou, Noninvasive,  
971 transient and selective blood-brain barrier opening in non-human primates in vivo, *Plos*  
972 *One*, 6 (2011) e22598.

973 [157] M.E. Downs, A. Buch, M.E. Karakatsani, E.E. Konofagou, V.P. Ferrera, Blood-Brain  
974 Barrier Opening in Behaving Non-Human Primates via Focused Ultrasound with  
975 Systemically Administered Microbubbles, *Sci Rep-Uk*, 5 (2015).

976 [158] A.K. Aslund, S. Snipstad, A. Healey, S. Kvale, S.H. Torp, P.C. Sontum, C.L. Davies, A.  
977 van Wamel, Efficient Enhancement of Blood-Brain Barrier Permeability Using Acoustic  
978 Cluster Therapy (ACT), *Theranostics*, 7 (2017) 23-30.

979 [159] B. Marty, B. Larrat, M. Van Landeghem, C. Robic, P. Robert, M. Port, D. Le Bihan, M.  
980 Pernot, M. Tanter, F. Lethimonnier, S. Meriaux, Dynamic study of blood-brain barrier  
981 closure after its disruption using ultrasound: a quantitative analysis, *J Cereb Blood Flow*  
982 *Metab*, 32 (2012) 1948-1958.

983 [160] Z.I. Kovacs, S.R. Burks, J.A. Frank, Concerning sterile inflammation following  
984 focused ultrasound and microbubbles in the brain, *P Natl Acad Sci USA*, 114 (2017)  
985 E6737-E6738.

986 [161] Z.I. Kovacs, S. Kim, N. Jikaria, F. Qureshi, B. Milo, B.K. Lewis, M. Bresler, S.R. Burks,  
987 J.A. Frank, Disrupting the blood-brain barrier by focused ultrasound induces sterile  
988 inflammation, *Proc Natl Acad Sci U S A*, 114 (2017) E75-E84.

989 [162] J. Silburt, N. Lipsman, I. Aubert, Disrupting the blood-brain barrier with focused  
990 ultrasound: Perspectives on inflammation and regeneration, *P Natl Acad Sci USA*, 114  
991 (2017) E6735-E6736.

992 [163] N. McDannold, N. Vykhodtseva, S. Raymond, F.A. Jolesz, K. Hynynen, MRI-guided  
993 targeted blood-brain barrier disruption with focused ultrasound: histological findings in  
994 rabbits, *Ultrasound Med Biol*, 31 (2005) 1527-1537.

995 [164] T. Sun, Y.Z. Zhang, C. Power, P.M. Alexander, J.T. Sutton, M. Aryal, N. Vykhodtseva,  
996 E.L. Miller, N.J. McDannold, Closed-loop control of targeted ultrasound drug delivery  
997 across the blood-brain/tumor barriers in a rat glioma model, *P Natl Acad Sci USA*, 114  
998 (2017) E10281-E10290.

999 \*\* A nice study showing how feedback can be used to control drug delivery by  
1000 sonopermeation

1001 [165] J.F. Jordao, C.A. Ayala-Grosso, K. Markham, Y. Huang, R. Chopra, J. McLaurin, K.  
1002 Hynynen, I. Aubert, Antibodies targeted to the brain with image-guided focused  
1003 ultrasound reduces amyloid-beta plaque load in the TgCRND8 mouse model of  
1004 Alzheimer's disease, *Plos One*, 5 (2010) e10549.

1005 [166] J.F. Jordao, E. Thevenot, K. Markham-Coultes, T. Scarcelli, Y.Q. Weng, K. Xhima, M.  
1006 O'Reilly, Y. Huang, J. McLaurin, K. Hynynen, I. Aubert, Amyloid-beta plaque reduction,  
1007 endogenous antibody delivery and glial activation by brain-targeted, transcranial  
1008 focused ultrasound, *Exp Neurol*, 248 (2013) 16-29.

1009 [167] S.B. Raymond, L.H. Treat, J.D. Dewey, N.J. McDannold, K. Hynynen, B.J. Bacskai,  
1010 Ultrasound enhanced delivery of molecular imaging and therapeutic agents in  
1011 Alzheimer's disease mouse models, *Plos One*, 3 (2008) e2175.

1012 [168] A. Burgess, Y. Huang, W. Querbes, D.W. Sah, K. Hynynen, Focused ultrasound for  
1013 targeted delivery of siRNA and efficient knockdown of Htt expression, *J Control Release*,  
1014 163 (2012) 125-129.

1015 [169] E.E. Konofagou, Neurorestoration of the nigrostriatal pathway through multiple  
1016 treatments with FUS-facilitated brain drug delivery, Abstract, 22nd European  
1017 symposium on Ultrasound Contrast Imaging, Rotterdam, (2017).

1018 [170] A. Burgess, C.A. Ayala-Grosso, M. Ganguly, J.F. Jordao, I. Aubert, K. Hynynen,  
1019 Targeted delivery of neural stem cells to the brain using MRI-guided focused ultrasound  
1020 to disrupt the blood-brain barrier, *Plos One*, 6 (2011) e27877.

1021 [171] Q. Huang, J. Deng, Z. Xie, F. Wang, S. Chen, B. Lei, P. Liao, N. Huang, Z. Wang, Z.  
1022 Wang, Y. Cheng, Effective gene transfer into central nervous system following  
1023 ultrasound-microbubbles-induced opening of the blood-brain barrier, *Ultrasound Med*  
1024 *Biol*, 38 (2012) 1234-1243.

1025 [172] E. Thevenot, J.F. Jordao, M.A. O'Reilly, K. Markham, Y.Q. Weng, K.D. Foust, B.K.  
1026 Kaspar, K. Hynynen, I. Aubert, Targeted delivery of self-complementary adeno-  
1027 associated virus serotype 9 to the brain, using magnetic resonance imaging-guided  
1028 focused ultrasound, *Hum Gene Ther*, 23 (2012) 1144-1155.

1029 [173] S. Wang, O.O. Olumolade, T. Sun, G. Samiotaki, E.E. Konofagou, Noninvasive,  
1030 neuron-specific gene therapy can be facilitated by focused ultrasound and recombinant  
1031 adeno-associated virus, *Gene Ther*, 22 (2015) 104-110.

1032 [174] L. Auboire, C.A. Sennoga, J.M. Hyvelin, F. Ossant, J.M. Escoffre, F. Tranquart, A.  
1033 Bouakaz, Microbubbles combined with ultrasound therapy in ischemic stroke: A  
1034 systematic review of in-vivo preclinical studies, *Plos One*, 13 (2018) e0191788.

1035 [175] R.M. Jones, L. Deng, K. Leung, D. McMahan, M.A. O'Reilly, K. Hynynen, Three-  
1036 dimensional transcranial microbubble imaging for guiding volumetric ultrasound-  
1037 mediated blood-brain barrier opening, *Theranostics*, 8 (2018) 2909-2926.



1038 [176] Z.S.I. Hu, H.L. McArthur, A.Y. Ho, The Abscopal Effect of Radiation Therapy: What  
1039 Is It and How Can We Use It in Breast Cancer?, *Curr Breast Cancer R*, 9 (2017) 45-51.  
1040 [177] W. Ngwa, O.C. Irabor, J.D. Schoenfeld, J. Hesser, S. Demaria, S.C. Formenti, Using  
1041 immunotherapy to boost the abscopal effect, *Nat Rev Cancer*, 18 (2018) 313-322.  
1042 [178] F. Hirabayashi, K. Iwanaga, T. Okinaga, O. Takahashi, W. Ariyoshi, R. Suzuki, M.  
1043 Sugii, K. Maruyama, K. Tominaga, T. Nishihara, Epidermal growth factor receptor-  
1044 targeted sonoporation with microbubbles enhances therapeutic efficacy in a squamous  
1045 cell carcinoma model, *Plos One*, 12 (2017).  
1046 [179] C.H. Fan, C.Y. Ting, H.L. Liu, C.Y. Huang, H.Y. Hsieh, T.C. Yen, K.C. Wei, C.K. Yeh,  
1047 Antiangiogenic-targeting drug-loaded microbubbles combined with focused ultrasound  
1048 for glioma treatment, *Biomaterials*, 34 (2013) 2142-2155.  
1049 [180] F. Yan, L. Li, Z. Deng, Q. Jin, J. Chen, W. Yang, C.K. Yeh, J. Wu, R. Shandas, X. Liu, H.  
1050 Zheng, Paclitaxel-liposome-microbubble complexes as ultrasound-triggered therapeutic  
1051 drug delivery carriers, *J Control Release*, 166 (2013) 246-255.  
1052 [181] Z. Kovacs, B. Werner, A. Rassi, J.O. Sass, E. Martin-Fiori, M. Bernasconi, Prolonged  
1053 survival upon ultrasound-enhanced doxorubicin delivery in two syngenic glioblastoma  
1054 mouse models, *J Control Release*, 187 (2014) 74-82.  
1055 [182] W. Luo, G. Wen, L. Yang, J. Tang, J. Wang, J. Wang, S. Zhang, L. Zhang, F. Ma, L. Xiao,  
1056 Y. Wang, Y. Li, Dual-targeted and pH-sensitive Doxorubicin Prodrug-Microbubble  
1057 Complex with Ultrasound for Tumor Treatment, *Theranostics*, 7 (2017) 452-465.  
1058 [183] Lipsman N, Ironside S, Alkins R, Bethune A, Huang YX, Perry J, Sahgal A, Trudeau  
1059 M, Hynynen K, M. T, Initial experience of blood-brain barrier opening for  
1060 chemotherapeutic-drug delivery to brain tumours by MR-guided focused ultrasound,  
1061 *Neuro-Oncology*, 19 (2017) vi275.  
1062 [184] A.V. Wamel, A. Healey, P.C. Sontum, S. Kvale, N. Bush, J. Bamber, C. de Lange  
1063 Davies, Acoustic Cluster Therapy (ACT) - pre-clinical proof of principle for local drug  
1064 delivery and enhanced uptake, *J Control Release*, 224 (2016) 158-164.  
1065  
1066