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# Ultrasound-Targeted Microbubble Destruction (UTMD) for Localized Drug Delivery into Tumor Tissue

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1     **Ultrasound-Targeted Microbubble Destruction (UTMD) for Localized Drug**  
2                                   **Delivery into Tumor Tissue**

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22

23 **Abstract**

24 Background: Ultrasound-targeted microbubble destruction (UTMD) is a type of ultrasound  
25 therapy, in which low frequency moderate power ultrasound is combined with microbubbles to  
26 trigger cavitation. Cavitation is the process of oscillation of gas bubbles causing biophysical  
27 effects such as pushing and pulling or shock waves that permeabilize biological barriers. *In vivo*,  
28 cavitation results in tissue permeabilization and is used to enable local delivery of nanomedicine.  
29 While cavitation can occur in biological liquids when high pressure ultrasound is applied, the use  
30 of microbubbles as cavitation nuclei in UTMD largely facilitates the induction of cavitation.  
31 UTMD is intensively studied for drug delivery into tumor tissue, but also for the activation of  
32 anti-tumor immune responses. The first clinical studies of UTMD-mediated chemotherapy  
33 delivery confirmed safety and efficacy of this approach.

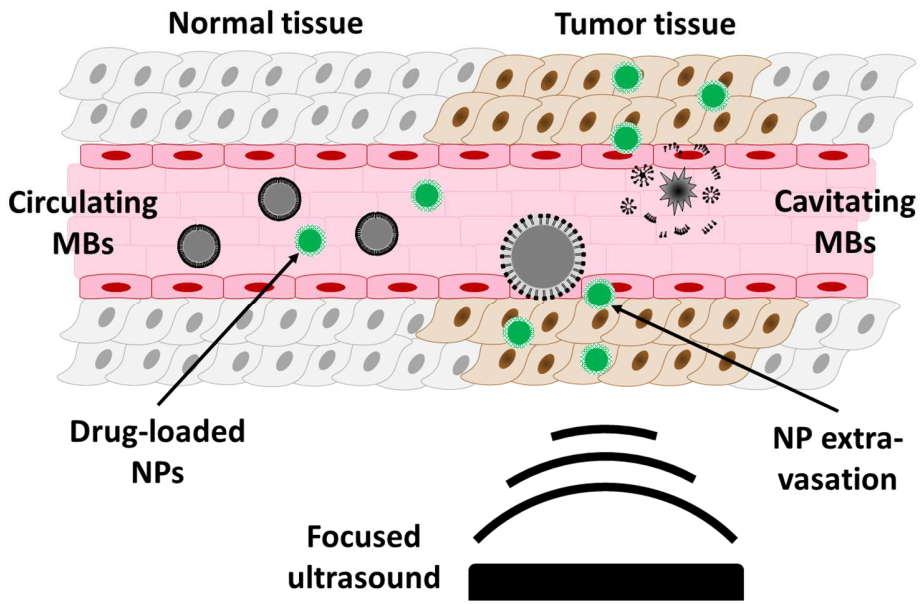
34 Aim: The present review summarizes ultrasound settings, cavitation approaches, biophysical  
35 mechanisms of drug delivery, drug carriers, and pre-clinical and clinical applications of UTMD  
36 for drug delivery into tumors.

37 Key points:

- 38 • UTMD is used for non-invasive localized drug delivery.
- 39 • Microbubbles serve as excellent cavitation nuclei.
- 40 • Stable and inertial UTMD cavitation effects contribute to tissue permeabilization.
- 41 • Drug carriers can be co-injected or directly coupled to microbubbles.
- 42 • A first clinical trial confirmed therapeutic efficacy in pancreatic cancer patients.

43

44 Graphical abstract:



45

46

## 47        **1. Localized drug delivery in cancer**

48            In intermediate and advanced cancer when surgery is not an option, patients are subjected  
49 to local radiotherapy, systemic chemotherapy, systemic molecular therapies, or combinations of  
50 these. Systemic therapies for cancer entail off-target delivery and toxicity side-effects which  
51 require the limitation of doses and decreased therapeutic efficacy [1]. Thus, local drug delivery  
52 approaches are required which either limit the drug's activity to the target site, release the drug at  
53 the target site, or activate the drug at the target site. Ultrasound-triggered drug delivery is  
54 mediated by thermal and/or mechanical effects including cavitation and radiation force. In this  
55 review, cavitation-mediated drug delivery is discussed. Ultrasound-targeted microbubble  
56 destruction (UTMD) causes cavitation and enhances permeability across natural barriers of  
57 tumors including vessel walls and cell membranes, resulting in spatio-temporally controlled  
58 enhanced drug delivery into tumors (**Figure 1**). Furthermore, mechanical ultrasound activities  
59 destabilize drug carriers and trigger drug release [2]. Ultrasound drug delivery is particularly  
60 attractive as it is non-invasive, enables the regulation of tissue penetration depth, and does not  
61 rely on ionizing radiation [2]. **In contrast to alternative drug delivery techniques, UTMD allows**  
62 **for targeted drug delivery.**

63            The present review aims at discussing the approach of UTMD for localized drug delivery  
64 into solid tumors. First, ultrasound settings are presented. Then, different approaches to achieve  
65 cavitation are elucidated. We then focus on the discussion of the biophysical mechanisms of  
66 UTMD. Different drug carrier systems are introduced. Finally, preclinical and clinical  
67 applications are summarized.

68

## 69        **2. Ultrasound settings**

70           Ultrasound enables the delivery of drugs at different tissue depths by modulation of  
71 ultrasound parameters such as frequency, duty cycle, mechanical index, and exposure time [2].  
72 For drug delivery, ultrasound frequencies range from kHz to MHz [16]. Lower frequencies  
73 enable deeper tissue penetration as attenuation effects are reduced. Furthermore, the frequency is  
74 adjusted to match the resonance frequency of ultrasound contrast agents such as microbubbles  
75 (MBs). Ultrasound intensity for drug delivery ranges from 0.3 to 3 W/cm<sup>2</sup> [16]. Lower intensities  
76 of US are used with longer pulse lengths or pulse repetition frequencies to achieve higher duty  
77 cycles and similar temporal average intensities than with high intensity ultrasound. The different  
78 parameters ensure fine-tuning according to the specific application such as tissue depth and MB  
79 type. The mechanical index is dependent on the peak negative pressure and center frequency and  
80 proportional to the ultrasound intensity. The FDA predefines the limit of the mechanical index to  
81 1.9 for clinical applications to minimize tissue damage [22]. Duration of ultrasound exposure is  
82 fixed to provide sufficient time to induce and maintain cavitation and tissue permeabilization  
83 while preventing tissue heating [16].

84           Focused and non-focused ultrasound transducers have been used for drug delivery in  
85 previous studies [23]. Non-focused ultrasound transducers cover bigger tissue volumes at once,  
86 which can accelerate the therapy protocol. However, cavitation is not limited to the tumor volume  
87 but can occur all along the acoustic ultrasound beam. Focused ultrasound transducers are  
88 commonly used for drug delivery as they ensure spatially controlled cavitation which is limited to  
89 a few millimeters [23]. While this guarantees specifically localized drug delivery, it brings along  
90 the drawback of longer treatment protocols to electronically steer or mechanically displace the  
91 transducer in order to insonify the whole tumor volume.

92           Image guidance ensures correct positioning of the therapy transducer to reach the target  
93 tissue. Image guidance further enables assessment of MB cavitation in real-time as MBs appear

94 as contrast-enhanced regions prior to cavitation and the signal is lost after MB destruction [24].  
95 Thus, image-guidance renders drug delivery more reliable and efficient. However, imaging does  
96 not allow tracking drug delivery as drugs diffuse differently from MBs.

97

### 98 **3. Cavitation nuclei**

99 Cavitation can be achieved with high pressure ultrasound and without the use of  
100 ultrasound contrast agents such as MBs. High pressure ultrasound results in generation and  
101 activation of gas nuclei in the tissue or vessels creating a cavitation cloud for pore formation,  
102 endocytosis, and vessel permeabilization [4]. Furthermore, ultrasound alone induces acoustic  
103 streaming which is weaker than microstreaming but can also trigger biological effects [4]. In  
104 these conditions, a high mechanical index is applied, which possibly limits the clinical translation  
105 of the approach. The use of MBs allows decreasing the mechanical index to diagnostic ultrasound  
106 levels as MBs are highly sensitive to ultrasound exposure and serve as excellent cavitation nuclei  
107 [2]. The cavitation response of MBs depends on their size and shell, and the ultrasound settings  
108 have to fit the MBs' resonance frequency. Further, the ultrasound pressure influences cavitation  
109 activity, and higher pressures have been shown to trigger successive MB implosions. The use of  
110 clinical-grade contrast agents, such as BR38 which was previously used for UTMD, further  
111 facilitates clinical translation [13–15]. Phase change nanodroplets are another option to provide  
112 cavitation nuclei which can be activated by low-intensity ultrasound, and in contrast to  
113 intravascular MBs, they can penetrate into the tissue and might further enhance the efficacy of  
114 drug delivery [16,17]. The use of molecularly targeted MBs, recognizing tumor endothelial  
115 markers, was shown to enhance drug delivery by creating direct contact with cell membranes and  
116 vessel walls [6]. Nanometer-sized ultrasound contrast agents, such as perfluorocarbon

117 nanoemulsions or echogenic liposomes with air pockets, have to be used for targeting of  
118 epithelial tumor markers as opposed to endothelial markers that can be reached by MBs [2].

119

#### 120 **4. Biophysical mechanisms of ultrasound drug delivery with microbubbles**

121 The combination of ultrasound with MBs for drug delivery is most widely studied and  
122 presents the focus of the present study. UTMD is based on the interaction of ultrasound and MBs  
123 to induce openings in vessels and membranes. Under low-pressure ultrasound, MBs expand and  
124 contract inversely proportional to the acoustic pressure waves, a process called stable cavitation.  
125 If the pressure is high enough, MBs cavitate non-linearly i.e. they expand more than they contract  
126 which leads to an increase in MB size until implosion and collapse (**Figure 2**). Low-intensity  
127 ultrasound-mediated stable cavitation and high-intensity ultrasound-induced inertial cavitation  
128 trigger different biophysical responses, which are discussed separately.

##### 129 ***Stable cavitation***

130 Stably oscillating MBs exert direct mechanical effects on adjacent biological barriers.  
131 They push and pull on surfaces and induce fluid jets, microstreaming, and shear stress (**Figure 3**)  
132 [3]. The shear stress intensity varies with the ultrasound settings between 100 Pa and 1000 Pa and  
133 is much higher than blood flow-associated shear stresses (0.1-4 Pa) [4]. Stable cavitation is  
134 mainly associated with formation of small pores and endocytosis [4]. As ultrasound can trigger  
135 pore formation, this type of tissue permeabilization is also called sonoporation. Small molecules  
136 enter cells through small pores while larger molecules are taken up by endocytosis. Pore  
137 formation requires direct contact between cell surfaces and MBs to mechanically disturb the  
138 membrane by pushing and pulling, or microstreaming which surrounds the oscillating MB [5].  
139 Interestingly, the use of targeted MBs that bind to cell surfaces requires lower ultrasound  
140 intensities to achieve the same membrane permeabilizing effects [6]. MBs are further modulated



141 by ultrasound waves through radiation forces which push MBs into the direction of the  
142 ultrasound beam. This phenomenon can potentially contribute to ultrasound-mediated drug  
143 delivery bringing MBs into contact with cell membranes and facilitating permeabilization [4].  
144 Radiation forces might also cause MB compression and pushing into cellular membranes thereby  
145 directly permeabilizing the cells [7]. MBs were also observed to be internalized into cells  
146 possibly through fusion of cell membrane and MB shell [8]. Sonoporation-mediated membrane  
147 pore formation is reversed when ultrasound is switched off [4]. Thus, membrane permeabilization  
148 duration is dependent on the duration of the ultrasound treatment, which has to be taken into  
149 consideration for effective delivery of drugs. Besides these mechanical effects, stably cavitating  
150 MBs in close vicinity of cells produce chemical stress leading to formation of free radicals and  
151 reactive oxygen species, which contribute to the permeabilization of cell membranes [4]. Further,  
152 stable cavitation was shown to mediate membrane hyperpolarization through activation of ion  
153 channels mediating calcium influx and potassium efflux [4].

#### 154 *Inertial cavitation*

155 Under high-intensity ultrasound exposure, the oscillation amplitude of MBs increases  
156 with every cycle and ultimately results in MB collapse; a process called inertial cavitation. Upon  
157 collapse, MBs fragmentize into several smaller MBs which in turn undergo inertial cavitation.  
158 Collapsing MBs exert high shear stresses, and shock waves, which rupture surfaces and induce  
159 pores (**Figure 3**) [9]. MBs in contact with cell membranes undergo asymmetrical collapse which  
160 results in liquid jet formation [10]. Shock waves and liquid jets not only perforate adjacent cell  
161 membranes but have the energy to permeabilize blood vessels [4]. Ultrasound settings such as  
162 pressure, exposure time, and pulse repetition frequency determine the size of induced pores  
163 which affects the delivery of larger drugs or drug carriers [11]. Indeed, UTMD-mediated  
164 membrane pore formation allows cytosolic delivery which is important for gene therapy as DNA

165 can be degraded in the endocytic uptake pathway [2]. In addition to the biophysical effects  
166 described for stable cavitation, inertial cavitation triggers harsh mechanical and chemical insults  
167 causing membrane perforation and vessel permeabilization. Inertial cavitation enhances drug  
168 delivery even when MBs are not directly interacting with cell surfaces as its effects act over  
169 longer distances [4]. In comparison to stable cavitation, inertial cavitation induces membrane  
170 pores of larger sizes ranging from hundreds of nanometers to a few micrometers [4].

171 Both stable and inertial cavitation were shown to trigger endocytosis-mediated uptake of  
172 larger drugs (150 – 500 kDa) while smaller drugs (4 – 70 kDa) entered cells through pores [12].  
173 Although, the exact mechanism triggering endocytosis after cavitation is not understood, it is  
174 speculated that microstreaming and acoustic streaming deform the plasma membrane, re-arrange  
175 the cytoskeleton, and activate mechano-sensors, and all of these processes contribute to  
176 endocytosis signaling (**Figure 3**) [4].

177

## 178 **5. Drug carriers**

179 In addition to the ultrasound settings and the type of cavitation nuclei, UTMD-mediated  
180 drug delivery is affected by drug administration, which can be performed by intra-tumoral,  
181 intraperitoneal, or intravenous injection [16]. Due to the invasiveness of intra-tumoral and  
182 intraperitoneal injections, and the heterogeneous delivery upon intra-tumoral administration, the  
183 intravenous administration route is usually preferred [16]. Drawbacks of the intravenous delivery  
184 are systemic toxicity and degradation, which have to be addressed with the preparation of  
185 appropriate drug carriers. There are generally two options for UTMD drug delivery: drugs can be  
186 encapsulated into drug carriers which will be co-injected with MBs, or drugs are loaded into MBs  
187 (**Figure 4**).

188 During MB synthesis, drug-loaded liposomes can be attached to the MB shell, negatively  
189 charged nucleic acids can be absorbed on a cationic MB surface, a second hydrophobic shell can  
190 be added to the MB membrane in order to provide a drug storage compartment, or hydrophobic  
191 drugs are directly inserted into the MB membrane [18]. These different loading strategies enable  
192 loading of different types and quantities of drugs, and sufficient drug delivery requires  
193 adjustment of injected MB concentrations [16]. **Direct coupling of drugs to MBs is considered a**  
194 **more effective drug delivery strategy because drugs are more proximal to vessels and membranes**  
195 **that are permeabilized by cavitating MBs.**

196 Alternatively, different types of drug carriers can be co-injected with MBs and used in  
197 combination with UTMD. Gold nanoparticles (NPs), silica NPs, polymer NPs, nanoemulsions,  
198 liposomes, and micelles were studied for UTMD [16,19]. PEGylated poly (lactic-*co*-glycolic  
199 acid) nanoparticles (PLGA-NPs), are larger therapeutic carriers (~110 nm), which are  
200 biocompatible, biodegradable, and FDA-approved [16]. They protect encapsulated drugs from  
201 clearance by the reticuloendothelial system, and allow for slow and prolonged release of drugs in  
202 the range of days to weeks so that they have more time to reach the target site [14,16]. The use of  
203 encapsulated chemotherapeutics further showed to reduce systemic toxicity, as observed with  
204 free doxorubicin while liposomal doxorubicin (Doxil) is better tolerable [20]. To ensure sufficient  
205 loading capacity, drug carriers have a size range of 90 to 300 nm and can be efficiently delivered  
206 by UTMD applying acoustic pressures below 1 MPa for small molecules (<1 nm) and high  
207 pressures at 5-6 MPa for larger drug carriers (>100 nm) [14]. Using PLGA-NPs, Willmann and  
208 colleagues were able to achieve the delivery of microRNAs for colon cancer and hepatocellular  
209 carcinoma (HCC) therapy in pre-clinical studies [14,15,21].

210

## 211 **6. Applications**

212 UTMD is applied for delivery of drugs ranging from nucleic acids, to proteins, and  
213 chemotherapies. It is currently intensively studied for blood brain barrier opening and targeted  
214 drug delivery in the central nervous system which is naturally well protected against external  
215 factors hindering therapy by systemic administration [25]. UTMD-triggered drug delivery was  
216 further studied in pre-clinical models of different types of cancer including brain, liver,  
217 pancreatic, breast, and ovarian cancers [26–30]. Sonochemotherapy describes ultrasound-  
218 mediated delivery of chemotherapy and pre-clinical studies showed positive therapy outcomes  
219 when UTMD resulted in increased intra-tumoral drug concentrations [31]. UTMD-mediated drug  
220 delivery further proved to decrease accumulation of drugs in healthy tissues such as heart, spleen,  
221 liver, lung, and kidney [29]. Though, UTMD can affect tumor perfusion as strong cavitation  
222 causes not only blood vessel permeabilization but also damage, and this can cause vascular  
223 shutdown which counteracts UTMD drug delivery [31,32]. Thus, control of cavitation  
224 localization and dose is required to obtain optimal drug delivery efficacy.

225 Ultrasound is further used for *in vitro* delivery of immunomodulatory agents such as  
226 tumor antigen-encoding and dendritic cell-stimulating mRNA to enable cancer immunotherapy  
227 [33]. Furthermore, ultrasound-mediated cavitation was shown to trigger tissue damage at the  
228 subcellular level thereby releasing different danger signals that elicit immune responses [34].  
229 Different studies showed that cavitation enabled penetration of dendritic cells, activated cytotoxic  
230 T cells, and Natural Killer cells into the tumor tissue [35–37]. Moreover, UTMD was applied to  
231 facilitate delivery of immuno-modulatory molecules or genes that encode tumor antigens and co-  
232 stimulatory factors [38,39]. Mechanical ultrasound without drugs decreased tumor growth and  
233 enhanced immune cell infiltration into subcutaneous solid tumors and across the blood brain  
234 barrier [40,41]. The degree of tissue destruction, which is dependent on ultrasound settings and  
235 tissue characteristics, is expected to contribute to the resulting biologic responses [42].

236 Researchers from Stanford University developed a novel approach based on ultrasound-  
237 guided delivery of molecular drugs encapsulated in NPs for HCC therapy. Their results showed  
238 to increase the concentration of delivered microRNA-loaded NPs up to 14-fold, the penetration  
239 depth of NPs up to 3-fold, and the concentration of delivered miRNAs up to 7.9-fold in tumor  
240 cells compared to treatment without ultrasound [14].

241 Recently a first clinical case study was performed based on sonochemotherapy. Five  
242 patients with pancreatic cancer were treated with ultrasound, MBs, and gemcitabine resulting in  
243 improved physical state and prolonged survival as the tumor size was transiently or even  
244 permanently decreased, or the tumor growth slowed down [43]. A human clinical trial using the  
245 same sonochemotherapy approach in inoperable pancreatic cancer patients confirmed safety and  
246 therapeutic efficacy as the median survival was doubled, and in five out of ten patients the tumor  
247 size decreased [44].

248

## 249 **7. Conclusions**

250 Overall, UTMD-mediated drug delivery proved feasible in multiple pre-clinical studies  
251 and in the first studies with human cancer patients. It is a versatile technique for targeted drug  
252 delivery into cancer enhancing local drug concentration and reducing off-side delivery and  
253 toxicity. UTMD is further studied for cancer immunotherapy enhancing anti-cancer immune  
254 responses by antigen release and immune cell infiltration. Developments in ultrasound  
255 technology, MB design such as molecular targeting with ligands, drug carrier preparation, and  
256 treatment protocols are expected to leverage this technique for improved cancer management.

257

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267

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403

404 **Figure Legends**

405

406 **Figure 1: Ultrasound drug delivery via ultrasound-targeted microbubble destruction**  
407 **(UTMD).** Microbubbles (MBs) are intravenously injected and circulate freely in the blood  
408 vasculature. Upon ultrasound exposure using a focused transducer, MBs in the tumor vasculature  
409 undergo cavitation, which leads to breakdown of cell junctions, cell membrane perforation, and  
410 tissue permeabilization. Co-injected drugs, such as drug-loaded nanoparticles (NPs), therefore  
411 penetrate more easily into the tumor tissue increasing the local drug concentration.

412

413 **Figure 2: Physical effects of ultrasound on microbubbles (MBs) and biophysical effects of**  
414 **MBs on the endothelium.** At low acoustic pressures, MBs oscillate linearly and undergo  
415 compression and expansion proportional to the pressure wave. At high acoustic pressures, MBs  
416 oscillate non-linearly: they expand faster than they compress and continue to grow until they  
417 collapse. Stably cavitating MBs exert forces on the adjacent endothelium: MBs can get pushed to  
418 the cell barrier by acoustic radiation force, microstreaming effects around the MBs hit cell  
419 membranes, and MB oscillation pushes and pulls on cells. Inertially cavitating MBs have even  
420 stronger effects on cells: shock waves disrupt cell junctions and liquid jets perforate cells.

421

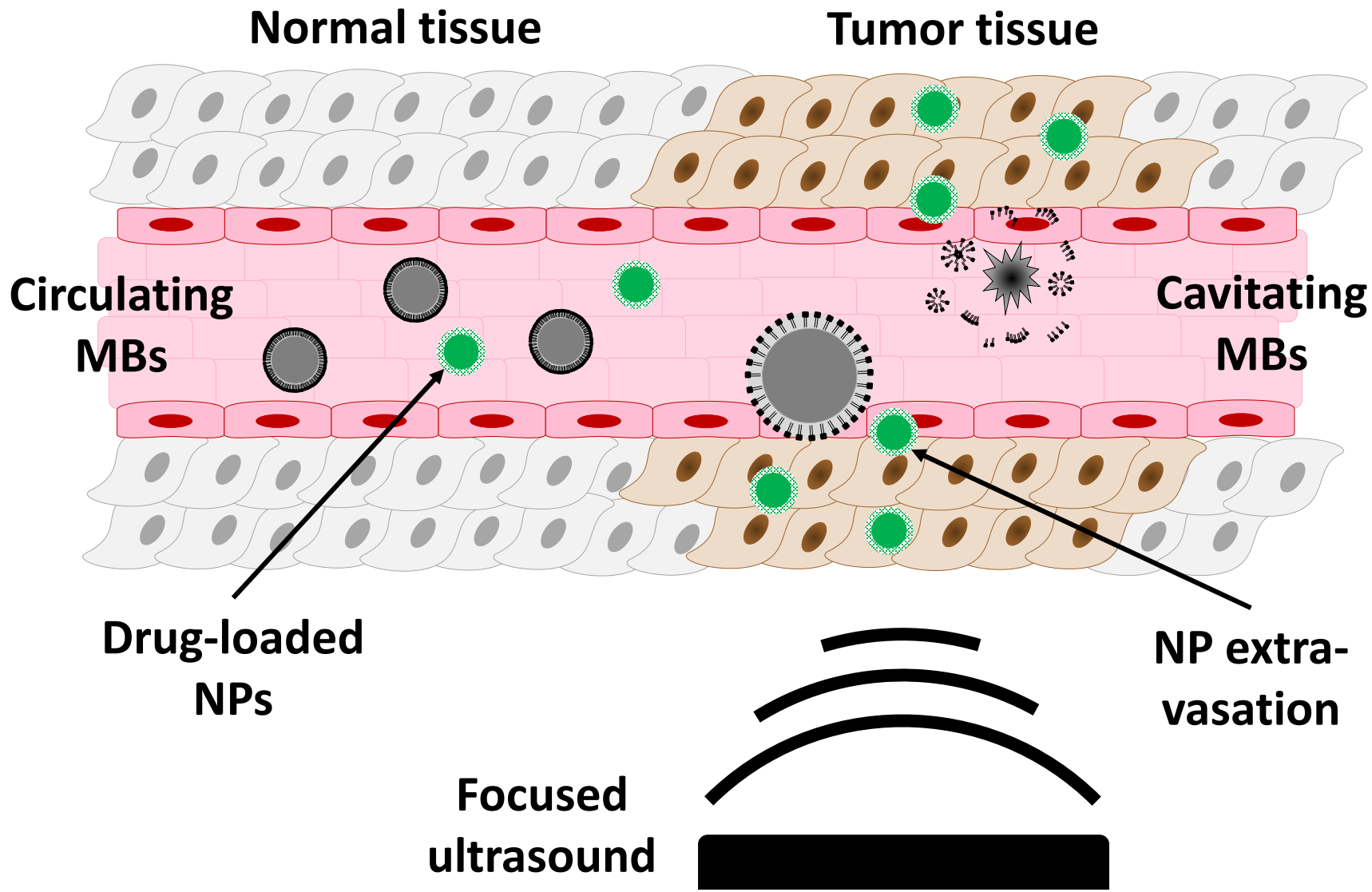
422 **Figure 3: Biological effects of stable ad inertial cavitation.** Stably and inertially cavitating  
423 microbubbles trigger mechanical effects such as pore formation which allows drug diffusion into  
424 the cell. Cavitation further produces reactive oxygen species interfering with ion channels or  
425 opening the membrane by lipid peroxidation. Stable cavitation induces microstreaming and shear  
426 stresses, which deform membranes and activate mechano-sensors. These cavitation-triggered  
427 processes contribute to endocytosis and exocytosis.

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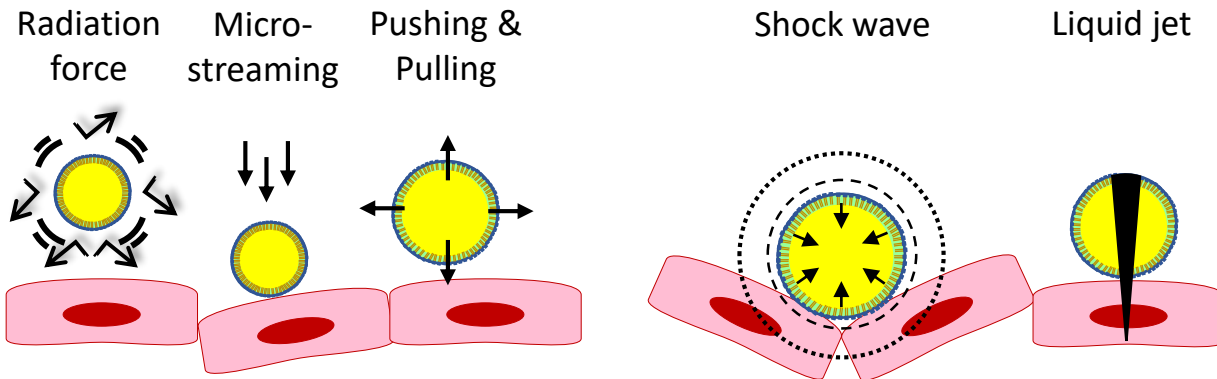
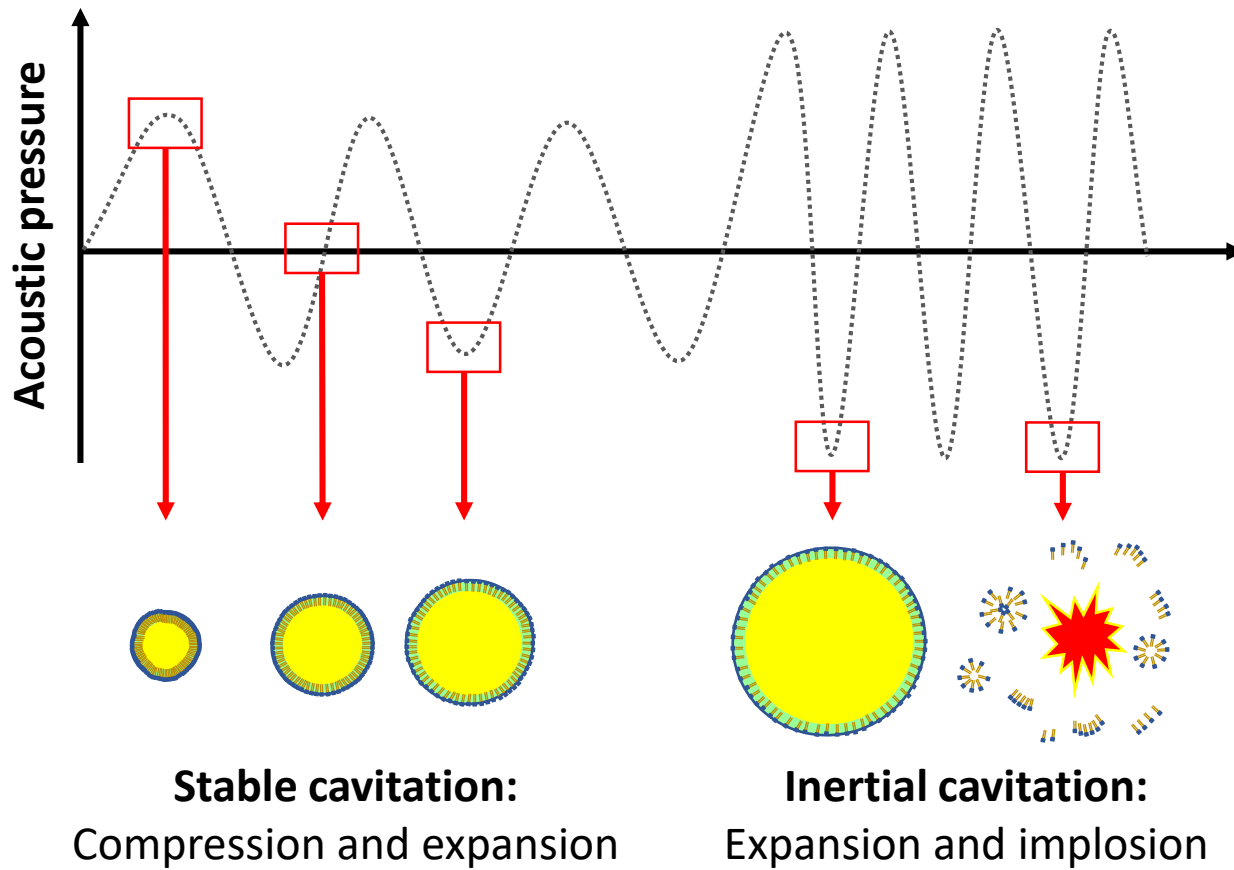
429 **Figure 4: Drug loading strategies for gas-filled microbubbles (MBs).** Lipid-, protein- or  
430 polymer-shelled MBs can be used for drug delivery. Drugs can be loaded directly into the shell,  
431 attached to the outside or included in an oil layer inside the MB.

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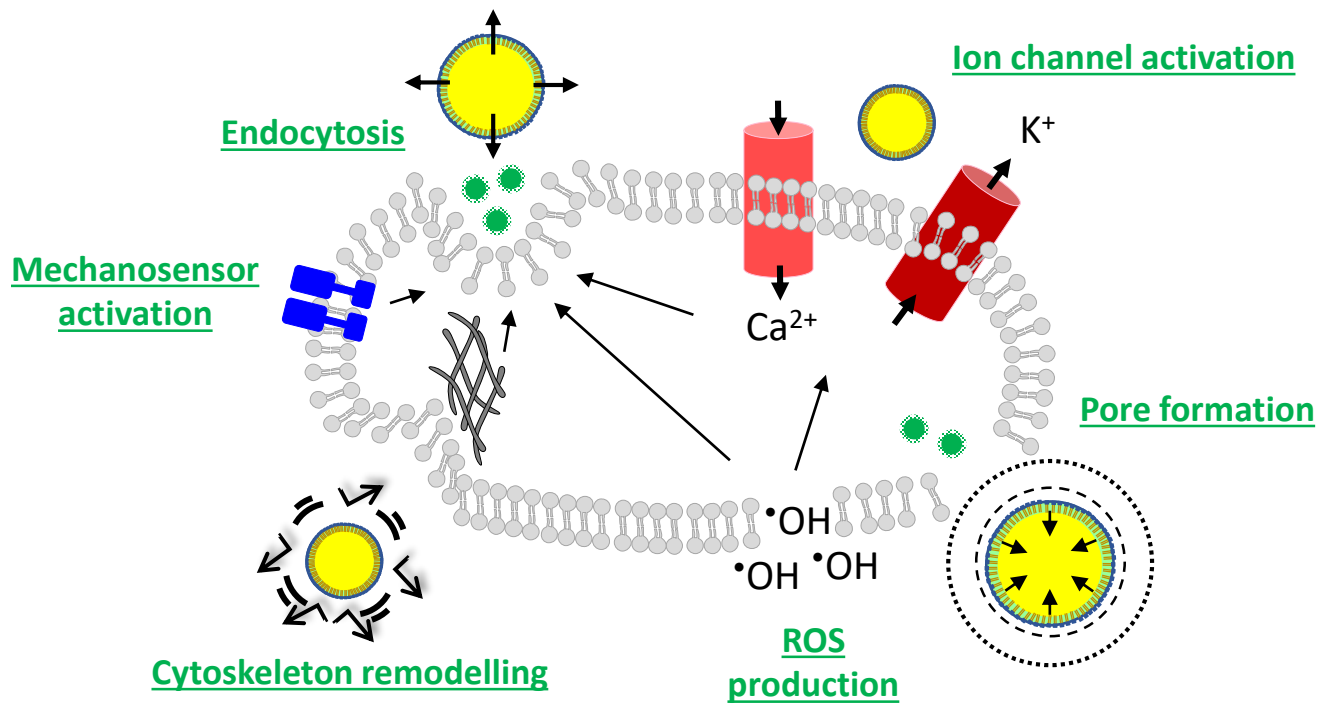


**Figure 1**

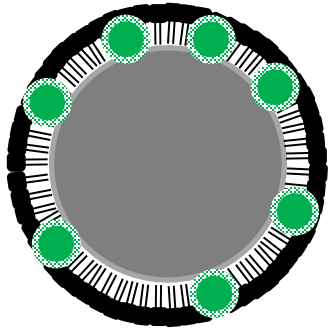


**Figure 2**

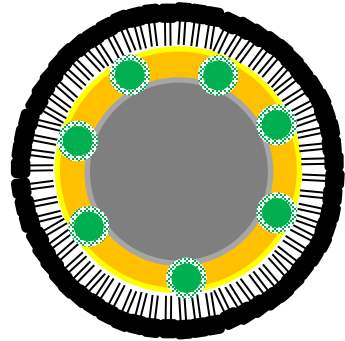




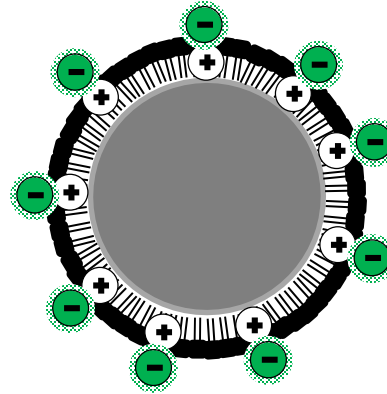
**Figure 3**



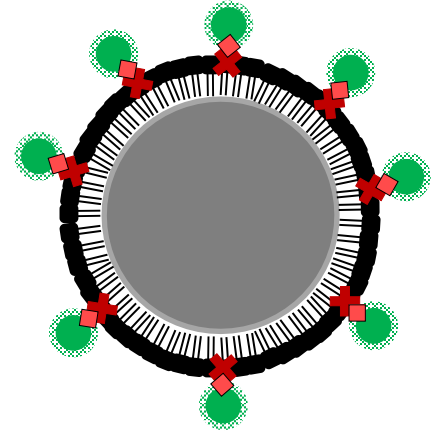
Lipid-shelled MB with drug in lipid membrane



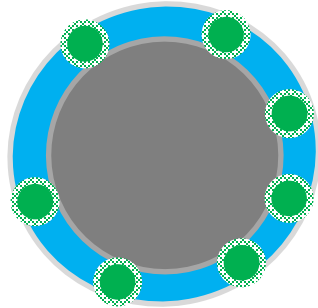
Lipid-shelled MB with drug in oil layer



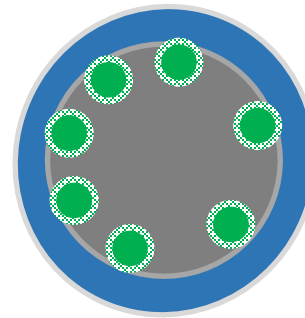
Positively charged lipid-shelled MB with negatively charged drug



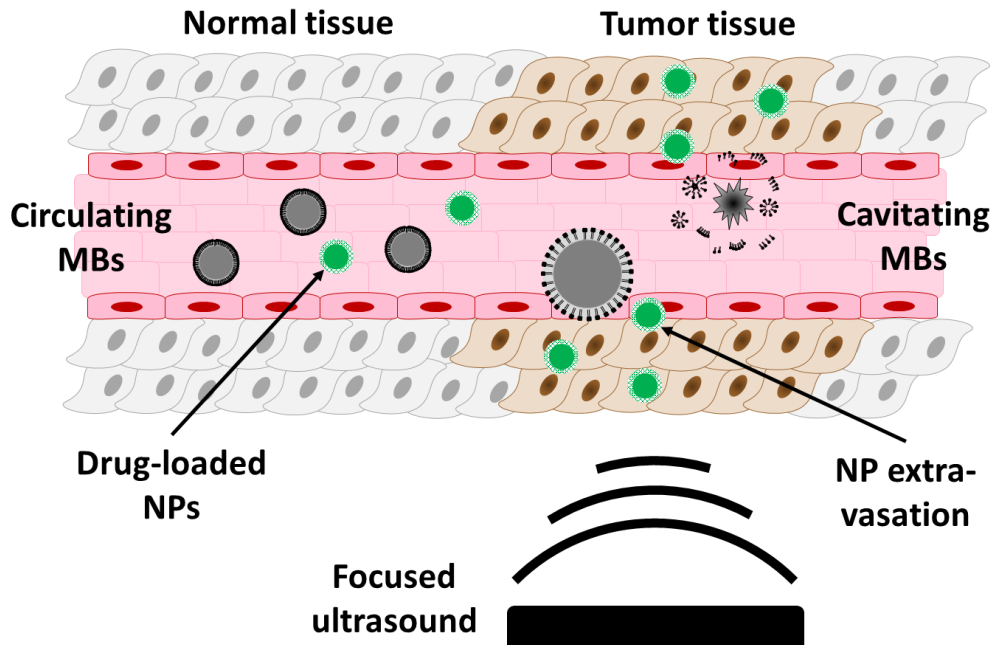
Avidinylated lipid-shelled MB with biotinylated drug



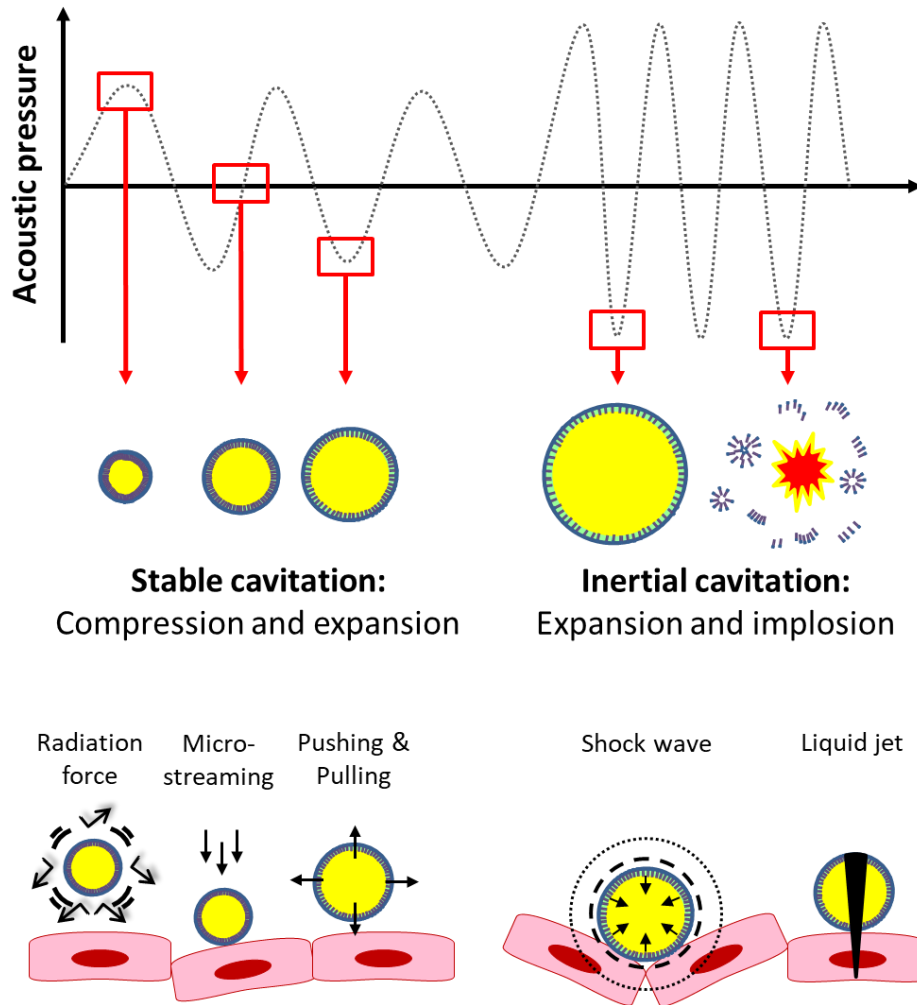
Protein-shelled MB with drug in protein layer



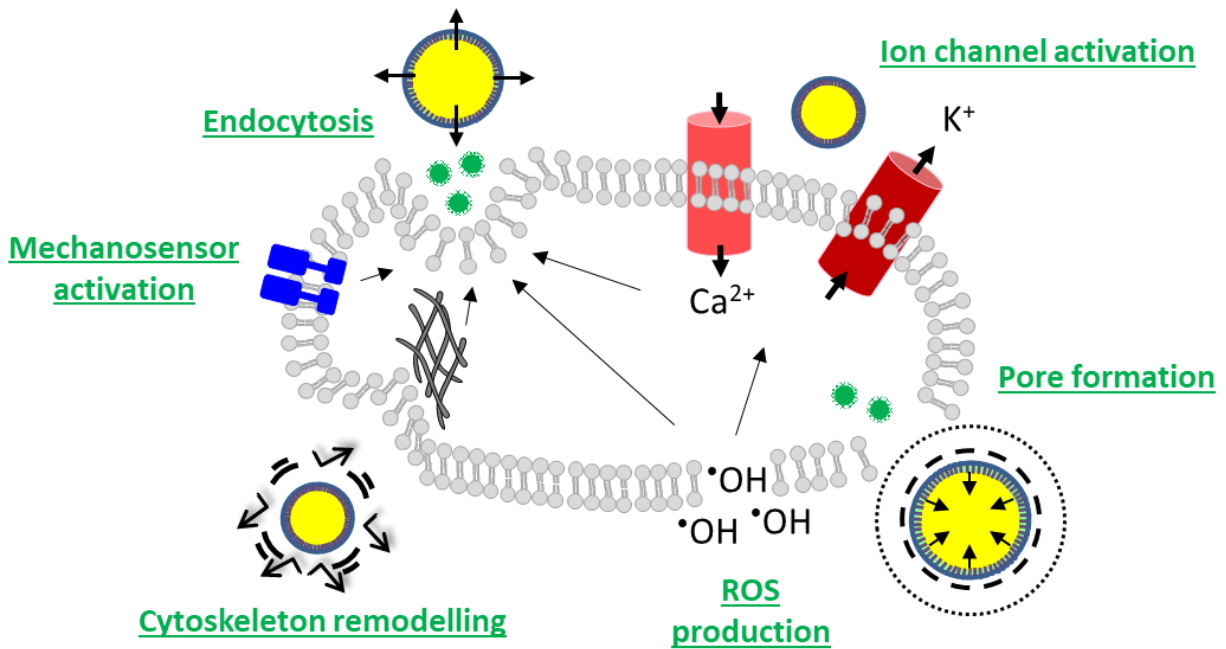
Polymer-shelled MB with drug attached to polymer



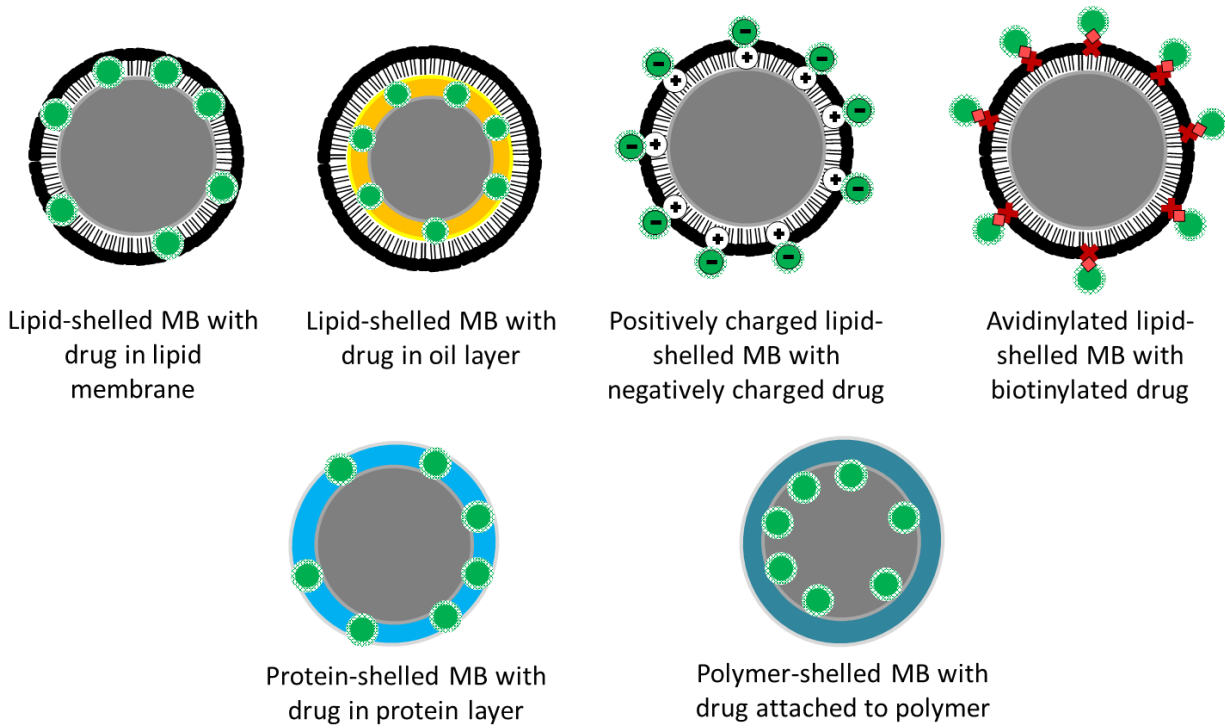
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