BCSJ

Document type: Review/Account

Account/Review for Life Chemistry

Ultrasound-Activated Nanomaterials for Therapeutics

Juan L. Paris*[1] and María Vallet-Regí*[2,3]

[1]Department of Life Sciences, Nano4Health Unit, Nanomedicine Group. International Iberian Nanotechnology Laboratory (INL), Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal

[2]Dpto. Química en Ciencias Farmacéuticas (Unidad Docente de Química Inorgánica y Bioinorgánica), Facultad de Farmacia, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), 28040-Madrid, Spain

[3]Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

E-mail: juan.paris@inl.int (J. L. Paris), vallet@ucm.es (M. Vallet-Regí)

Received: November 30, 2019; Accepted: December 4, 2019; Web Released: December 12, 2019

Juan L. Paris
Juan L. Paris received his Ph.D. from Universidad Complutense de Madrid (Spain) in 2017. During his PhD he worked on ultrasound-responsive mesoporous silica nanoparticles for biomedical applications. He is currently a *Ramón Areces* Postdoctoral Fellow at the International Iberian Nanotechnology Laboratory (INL), located in Braga (Portugal). His current research is focused on stimuli-responsive self-assembled soft nanomaterials for drug and gene delivery.

María Vallet-Regí
María Vallet-Regí received her Ph.D. from Universidad Complutense de Madrid in 1974. María Vallet-Regí is a chemist, scientist, and professor at Universidad Complutense de Madrid, Spain. She is recognized as a pioneer in the field of ceramic materials applied to medicine. She was the pioneer who suggested introducing drugs into the pores of MSMs, which inspired thousands of publications worldwide involving MSNs for drug delivery. Her current research interests are focused on developing nanomedicines for treating different diseases, such as bone infection, osteoporosis or bone cancer.

Abstract

Ultrasound has attracted much attention in recent years as an external stimulus capable of activating different types of nanomaterials for therapeutic application. One of the characteristics that makes ultrasound an especially appealing triggering stimulus for nanomedicine is its capacity to be non-invasively applied in a focused manner at deep regions of the body. Combining ultrasound with nanoparticles, different biological effects can be achieved. In this work, an overview of the four main types of inducible responses will be provided: inducing drug release, producing ultrasound-derived biological effects, modifying nanoparticle biodistribution and developing theranostic agents. Several examples of each one of these applications are presented here to illustrate the key concepts underlying recent developments in the discipline.

Keywords: Ultrasound | Nanomedicine | Stimuli-responsive

1. Introduction

Stimuli-responsive nanomaterials for biomedicine have attracted great attention in recent years. This type of material can produce different responses (commonly involving release of a drug) when exposed to a certain stimulus.[1,2] The triggering stimulus can be internal, if the pathology of interest is characterized by changes in pH,[3,4] redox potential[5,6] or presence of certain enzymes;[7] or it can be something externally applied by the physician, such as light,[8–11] magnetic fields[12–15] or ultrasound (US).[16] In this work, we will focus on US-responsive nanomaterials and what different responses can be engineered into nanomaterials for biomedicine. In order to be able to properly describe the progress made in this topic, we will first have to define some basic concepts related to US and its effects in biological environments.

US can be defined as an acoustic (mechanical) wave whose frequency is above the human hearing limit (20 kHz).[8] The US wave can be defined as a function of various parameters, such as frequency, power, intensity or pressure. The speed of US divided by its wavelength results in the frequency, which is the most common parameter to describe a US wave. The velocity of the US in water, which is the medium by which it is to be transmitted in a living organism, is 1480 m/s.[17] Depending on its frequency, it can be divided into low frequency US (below 1 MHz) or high frequency US (above 1 MHz).[8] US power can be expressed in watts (W), although in the clinical environment the concept of intensity (expressed in W/cm^2) is more commonly used.[18–20] Since it is a mechanical wave, the pressure generated by the US application is also commonly used to describe the US conditions applied.[21]

The use of US in the clinic is widely used for both diagnostic and therapeutic purposes.[22] It has been used for a multitude of applications, such as the generation of hyperthermia,[23] opening of the blood-brain barrier,[24] sonoporation,[25] immunostimulation,[26] diagnostic imaging[27] and physiotherapy,[19] among many others. For its diagnostic use, high frequencies (generally above 3 MHz) are usually employed, working at low intensities.[28] On the other hand, for therapeutic application, lower frequencies are used, generally working at a higher intensity.

The generation of US usually takes place using transducers constituted by piezoelectric crystals, capable of converting an electrical signal into a mechanical wave that will be transmitted by a fluid.[16] The use of transducers with curvature allows the generation of a focal point at a certain distance from the source of high frequency US. This makes it possible to apply greater stimulus intensity to the point of interest at a certain depth within the body in a non-invasive manner, minimizing the intensity of the US to which the surrounding healthy tissues will be exposed.[23] The ability to focus the US at a certain depth has led to the development of therapies using high-intensity focused US (HIFU). The use of HIFU is proposed as an alternative to surgery in some cases of internal tumors, by destroying tissue at the US focal point.[28]

The high penetration capacity of the US and the possibility of applying it in a focused manner constitute great advantages over other widely studied external stimuli, such as light. The biological effects of US can be divided into thermal effects and mechanical effects.[23] However, it is worth mentioning that both types of effects will occur simultaneously, and that in most cases, separating the two is a complex task, as discussed below.

Thermal effects: As a US wave propagates through a tissue, some of its energy can be absorbed in the form of heat, increasing the temperature in the area.[23] Thus, US-generated hyperthermia can be used for ablative cancer therapies or to activate temperature-sensitive nanomaterials.[29,30] Thermal effects are directly related to the frequency of US used: higher frequencies produce a higher temperature rise when transmitted through tissues.[19]

Mechanical Effects: The mechanical effects of the US can be divided into non-cavitational effects and acoustic cavitation.[31] Among the former, the most common is acoustic streaming, which can be defined as the generation of movement or flow in a fluid as a result of its exposure to the mechanical waves of the US.[32] Acoustic cavitation, on the other hand, is due to the interaction of gas bubbles in a fluid with the US mechanical waves to which it is exposed.[33,34] It can be divided into three stages, resulting in stable cavitation or unstable cavitation (also referred to as inertial cavitation). First, exposure to mechanical waves induces the formation of small gas bubbles in the fluid. The interaction of these microbubbles with US waves causes them to oscillate in size, expanding and compressing in the positive and negative pressure phase, respectively (stable cavitation).[34] Finally, if the stimulus reaches sufficient intensity, exceeding a threshold size in the negative pressure phase induces the catastrophic collapse of the same in the positive pressure phase (inertial cavitation).[21] This implosion of the gas microbubble produces extreme conditions in the local environment (at the nanoscale), reaching very high pressures and temperatures of the order of 5000 K, which makes it difficult to separate thermal and mechanical effects.[8] These extreme conditions can result in the generation of light (sonoluminescence)[35] and the formation of reactive oxygen species (ROS) due to pyrolysis of water molecules.[31] The mechanical index (MI) is a parameter that indicates the probability of inertial cavitation in a fluid exposed to US.[36] It is defined as the negative pressure peak generated divided by the square root of the US frequency used. MI values greater than 0.7 indicate a high probability of inertial cavitation. Therefore, the lower the frequency used, the greater the probability of cavitation.

The generation of cavitation is considered as one of the most promising applications of the use of US in biomedicine, since it can generate a great multitude of biological effects. It is believed to be involved in the permeabilization of the cell membrane by US (sonoporation),[25] and can be used to promote the penetration of drugs and nanoparticles.[21] However, the pressure required to exceed the cavitation threshold *in vivo* may be too great to be used safely without damaging healthy tissue.[36] Therefore, the use of cavitation nuclei capable of lowering the pressure threshold required to produce acoustic cavitation appears to be a convenient option for biomedical application.[34] Among the most commonly used cavitation agents are micrometric lipid bubbles, which are already used in clinics as US contrast agents,[37] submicrometric phase change droplets[38] and submicrometric polymer particles with the ability to stabilize gas nanobububbles.[39,40]

Finally, it is worth mentioning that the thermal and mechanical effects of the US, and especially the phenomenon of inertial cavitation, can be used to accelerate or induce chemical reactions (sonochemistry).[41–46] The induction of chemical reactions in the presence of inertial cavitation may be due to the generation of reactive oxygen species that initiate a chain of subsequent reactions, to heating at the nanoscale in the vicinity of imploding bubbles, to mechanical effects such as acoustic microstreaming (also associated with cavitation), or to a combination of all these effects.

The US thermal,[47] mechanical[48] and chemical[49] effects can be used to develop different intelligent materials capable of triggering a response when exposed to stimuli. Thus, liposomes,[16,29,50,51] micelles,[42,49,52] polymeric particles[53] and hybrid particles[54–56] sensitive to US have been developed.

When combining these smart nanomaterials with US application, different effects can be obtained. The main goal of this work is not to collect a comprehensive list of all the research done in US-responsive nanoparticles, but to provide an over-

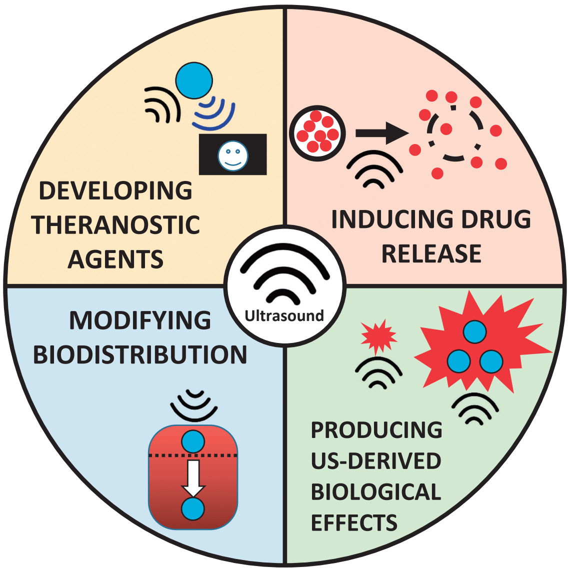

Figure 1. Graphical summary of the parts in which the present article is divided.

view of the main objectives with which US can be employed in the context of nanomedicine, with some recent examples presented to illustrate each of the concepts introduced. Accordingly, the main text of the article has been divided into four sections, corresponding to said objectives: Inducing drug release, producing US-derived biological effects, modifying biodistribution and developing theranostic agents (Figure 1).

2. Inducing Drug Release

The most studied triggered behavior in stimuli-responsive nanomaterials for biomedical application is induced drug release from nanocarriers.[16] US-triggered drug release systems can be classified by their type of release profile (single *vs* multiple-triggered systems), the type of nanoparticles employed (liposomes, polymeric nanoparticles, silica nanoparticles, etc), their mechanism of activation (through thermal, mechanical or chemical effects of US), and their target therapeutic application (for example: cancer, pain management, antimicrobial, rheumatoid arthritis). US-responsive drug carriers have also been developed in particularly promising areas of nanomedicine, such as co-delivery of different molecules by a single carrier,[57] or delivery of large molecular weight therapeutics.[58] The type of release profile desired for a specific material is closely related to its target therapeutic application, since it will depend on factors such as whether the administration is systemic or local, and the drug pharmacokinetics and toxicity, among others. Here, we will describe different nanosystems reported in the literature based on their type of release profile (single or multiple-triggered systems), discussing in each case the target application and the designed activation mechanism.

When discussing triggerable drug release, two main types of profiles can be distinguished: single-triggered release and multiple-triggered release. In single-triggered systems, the drug carrier is found in a closed configuration when administered, and after a certain lag time, material exposure to a certain stimulus (like US in the cases shown here) induces an irreversible change to an open configuration, which leads to the complete release of the loaded drug (although this release process can take a significant period of time to be completed). In multiple-triggered systems on the other hand, the exposure to the activating stimulus induces the release of only part of the loaded dose, enabling further triggering events that will each produce release of the drug. Often, in these systems, the dosage of released drug can be controlled by modifying the duration and/or intensity of the triggering stimulus, so in practice, if the stimulus is intense enough or is applied for a lengthy period of time, a material with multiple-triggered release capacity can be employed as a single-triggered system, by forcing the release of all the loaded drug at once.

2.1 Single-Triggered Drug Release Systems. The main rationale behind single-triggered drug-release nanosystems is that controlling the spatiotemporal distribution of free drug in systemically-administered nanotherapeutics might produce benefits as both increasing efficacy and reducing undesired side-effects. This is because preventing premature drug release during nanocarrier circulation throughout the organism would limit its action in undesired off-target organs, thus limiting dose-limiting toxicities. Then, the possible increase in effective administered dose in the target tissue could increase its therapeutic effect. A clear example of this kind of strategy can be seen in one of our recent works, where tumor-tropic mesenchymal stem cells were filled with chemotherapeutic drug-loaded single-US-triggerable mesoporous silica nanoparticles.[56] In this system, the mesenchymal stem cells would act as vehicles carrying the drug-loaded nanoparticles towards tumors. However, this migration process will normally take a few days, during which the survival of the carrier cells must be ensured. Premature release of the chemotherapeutic drug doxorubicin could compromise their viability and migration capacities, so we introduced a US-responsive gatekeeper on the nanoparticle surface to prevent it. However, once in the target tumor region, the maximum possible dose of drug should be released to produce the maximum possible effect. Therefore, a single-triggered system was ideal here, which we achieved by using an irreversible opening mechanism based on a US-induced reaction that induces a conformation change in the polymeric gatekeeper employed.

The most eminent example of US-triggered release nanosystems is probably the use of thermosensitive liposomes in combination with HIFU to produce the temperature increase.[29] These thermosensitive liposomes are obtained by carefully designing the lipid bilayer so that its transition temperature is slightly above physiological temperature, and in the hyperthermia region. By increasing the surrounding temperature slightly above the transition temperature of the lipids, a fast release of the loaded drug is produced. It must be highlighted that the combination of HIFU with the most clinically-advanced thermosensitive liposome (Thermodox®) is currently undergoing clinical trials, which have so far shown great promise in this strategy.[59] Besides thermosensitive liposomes, many other nanosystems have been described for the US-induced release of small molecule drugs, mainly in the context of anticancer therapy, by designing different activation mechanisms that take advantage of the different biological effects of US. As an example of non-thermosensitive US-responsive liposomes,

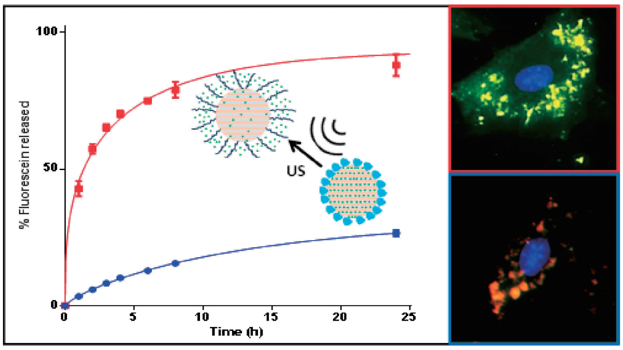

Figure 2. US-induced fluorescein release from hybrid mesoporous silica nanoparticles under suspension in PBS (left) or inside cells *in vitro* (right). Reprinted with permission from ref 54. Copyright 2015 American Chemical Society.

Schroeder *et al.* described a system capable of releasing cisplatin when exposed to low frequency US.[50] However, since low frequency US is difficult to focus, its use would be limited to superficial tumors. As we will see below, later works by other authors have described high frequency-responsive systems based on different activation mechanisms.

After liposomes, polymeric nanoparticles have probably been the most successful type of US-responsive nanocarriers for anticancer drug delivery. Among the main examples of this line of work, we can highlight the pioneering work in which Xuan *et al.*[52] described a polymeric micelle that could be activated by HIFU through the induction of a chemical reaction with one of the monomers employed. The conversion of hydrophobic THPMA into hydrophilic methacrylic acid displaced the lower critical solution temperature (LCST) of the copolymer employed, inducing a conformation change and releasing the drug. We later employed the same strategy to prepare hybrid polymer-grafted mesoporous silica nanoparticles[60] for anticancer drug release (Figure 2).[54–56,61] In a later work, Li *et al.* developed another micellar formulation based on block copolymers obtained by click-chemistry, in which ester bonds in the junction points of the two blocks are cleaved by HIFU, releasing a molecule loaded in the core of the micelles.[42] Finally, it is worth mentioning that this kind of sonochemical strategy can be employed to obtain micelle nanocarriers that can respond to several different stimuli, by including the appropriate chemical moieties in the polymer structure. As an example of this, Lin *et al.* developed a triple-responsive block copolymer that could respond to temperature, US and pH.[6] These multiple-stimuli responsive systems provide great design versatility, enabling fine-tuning the nanocarrier for specific therapeutic applications.

US-triggerable drug-releasing formulations can also be designed to be activated by different mechanisms than heat generation or sonochemistry. For example, Lee *et al.* developed a methotrexate-releasing nanosystem based on bursting gas bubbles generated from a chemical reaction.[62] In this strategy, nanoparticles are first used to deliver luminol, which reacts with ROS in cancer cells generating N_2 bubbles. These gas bubbles are then burst employing US, what induces release of methotrexate from the folate-conjugated bovine serum albumin nanoparticles. Core-shell particles made of mitoxantrone-encapsulating PLGA coated by liposomes can also be activated by the mechanical effects of US to release the loaded cargo.[63]

In the context of non-cancer therapeutic scenarios, Airan *et al.* have recently described a nanoemulsion system capable of releasing propofol when exposed to focused US.[64] With this nanosystem, the authors were capable of inducing therapeutic effects in an acute rat seizure model by applying extracranial focused US. On a different application, Zhu *et al.* have recently reported targeted and PEGylated perfluoropentane-based nanodroplets for dexamethasone delivery in a rheumatoid arthritis model.[65] These particles significantly decreased inflammation of joints when employed in combination with US.

2.2 Multiple-Triggered Release Systems. Despite the many advantages that single-triggered DDS can provide to different therapeutic applications, in many pathological conditions what would be desirable is the capacity to produce several drug release events. In such conditions, employing an external triggering event (such as the application of US) will also enable adapting the released dose and/or its timing to the changing needs of the patient. A clear example of this kind of strategy can also be found in one of our recent publications. In this case, the chosen application of our DDS was in the context of perioperative pain management.[51] Under this scenario, our goal was to develop a locally-injectable anesthetic-releasing liposomal formulation that could provide an initial effect that would take place during the surgical procedure, followed by a period of time in which the patient could control the amount of free anesthetic in the area. The main advantage of this kind of strategy in the clinical setting derives from the common motor block that is associated to the sensory block produced by local anesthetics, which for example eliminates the patient's capacity to move an affected limb. Providing the patient with control over the amount of free anesthetic the injected region would allow them to decide how much pain could be acceptable while still allowing a certain level of limb movability. To achieve this goal, we employed liposomes containing both a sonosensitizer and an unsaturated lipid. When exposed to US, the sonosensitizer would generate reactive oxygen species, which would induce the peroxidation of part of the unsaturated lipid in the bilayer, changing its permeability and inducing the release of part of the loaded anesthetic tetrodotoxin. When combined with dexmedetomidine-loaded liposomes, a maximum of 5 triggered anesthetic events could be achieved, even though the duration of the therapeutic event was reduced with each successive insonation. More recently, Gao *et al.* have reported a system based on multiple-triggerable hollow mesoporous organosilica nanoparticles capable of releasing bupivacaine when exposed to US, producing long-lasting analgesia.[66]

3. Producing US-Derived Biological Effects

The thermal, mechanical and biological effects of US can be directly used to generate a therapeutic effect in different pathological conditions. The combination of US with nano- or micro-particles can be used to amplify these effects, improving therefore the therapeutic efficacy that could be obtained with US alone.

The most obvious synergy between nano- or micro-particles and US is to enhance the effect of the mechanical effects of

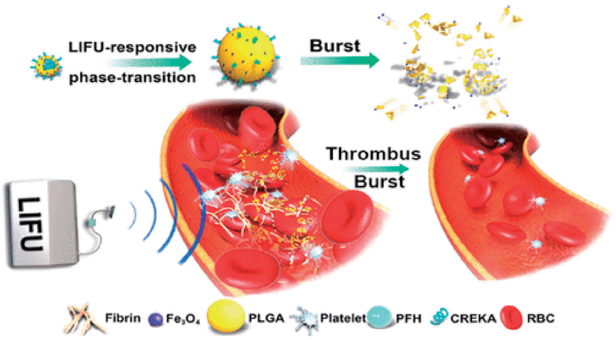

Figure 3. Conceptual representation of the use of phase-change perfluorohexane-derived nanodroplets for US-induced thrombolysis. Reprinted with permission from ref 67. Copyright 2019 American Chemical Society.

US. Gas-containing of gas-stabilizing materials (such as lipid microbubbles, phase-changing nanodroplets and submicrometric gas-stabilizing particles) can act as cavitation nuclei, decreasing the pressure threshold necessary to achieve acoustic cavitation. In this way, some therapeutic effect associated to cavitation can be achieved at a pressure at which no undesired effects will be provoked in surrounding non-target tissues. As a clear example of this strategy, Zhong *et al.* have recently described the use of perfluorohexane-derived nanodroplets as thrombolytic agents (Figure 3).[67] The CREKA peptide included in the formulation enabled thrombus targeting, and the application of low intensity focused US leads to a robust decrease in the thrombus burden. Similar sonothrombolysis can be achieved employing other cavitation nuclei, such as microbubbles.[68] In another work, Ishijima *et al.* showed that after cell uptake, phase-change nanodroplets were capable of drastically reducing the necessary pressure threshold to induce cancer cell death.[69]

Another potential therapeutic application of US, in this case by employing its chemical effects, is what has been called sonodynamic therapy.[31] Sonodynamic therapy (SDT, named as an analogous strategy to photodynamic therapy or PDT) consists in the generation of toxic ROS as a consequence of US application, and has been proposed in the context of anticancer and antimicrobial therapies. In general, two components are needed for SDT: a sonosensitizer and US. A sonosensitizer is a component that can be excited by US to generate ROS, but it does not imply any cytotoxic effect in the absence of stimulation.[35] The use of nano- or micro-particles for SDT has been reported to improve its efficacy through various means.[70] For example, improved delivery of sonosensitizer to tumors can be achieved by taking advantage of the passive accumulation of sonosensitizer-modified gold nanoparticles.[71] Employing O_2 microbubbles carrying a sonsosensitizer could also improve the efficacy of SDT in hypoxic tumors, since they not only decreased the pressure necessary to generate cavitation (and therefore, to activate the sonosensitizer), but also because they were at the same time providing the oxygen necessary to generate ROS.[72] A similar concept was also developed by Chen *et al.*, who employed oxygen and sonosensitizer-delivering hollow mesoporous nanoparticles to improve SDT efficacy.[73] Later work also showed the possibility of simultaneously using the same microbubbles as carriers of anticancer drugs, enabling combination therapy and enhancing the therapeutic effect.[15,74] The work reported by Li *et al.* also described combined delivery of sonosensitizer and chemotherapeutic drugs, in this case employing hollow mesoporous organosilica nanoparticles.[75] Nomikou *et al.* also showed that nanoparticles containing both a photosensitizer and a sonosensitizer enabled dual PDT-SDT, which provided enhanced efficacy when compared to both therapeutic modalities in isolation.[76] In the context of antimicrobial therapy, Pang *et al.* have recently reported the development of bacteria-targeted nanoliposomes that can deliver high concentrations of sonosensitizer into the target bacteria, achieving sonodynamic elimination of multidrug-resistant bacteria.[77]

4. Modifying Biodistribution

US can also be employed to modify the biodistribution of nanoparticles, either at the tissue or cellular level. Two parameters are fundamental to take into consideration when designing this kind of strategy: the nanoparticle administration route (either local/topical or systemic) and the target tissue/cells.

Topical administration of nanoparticles can be considered when the target area to treat is located in the skin. However, nanoparticle penetration into the skin tissue after direct administration on its surface is usually poor.[78] US application after topical administration of nanoparticles provides a mechanical impulse that drives deeper nanoparticle penetration in the tissue.[78,79] Besides US application, certain nanoparticle characteristics, such as surface charge, were also seen to affect the penetration capacity of the nanoparticles in skin tissue.[78] In order to further improve this effect, combination of nanoparticles with cavitation nuclei (capable of decreasing the pressure needed to trigger acoustic cavitation) was shown to enhance the skin penetration of nanoparticles upon US exposure.[80] This strategy could be employed to deliver nanoparticles to relatively deep areas of the skin, where they could provide a significantly improved therapeutic effect.

In the context of systemically administered nanoparticles, US has been explored to achieve selective tissue biodistribution in two broad contexts: to induce selective extravasation of nanoparticles in desired tissue (mainly in solid tumors) and to enable nanoparticle crossing the blood-brain barrier (BBB).

Selective nanoparticle extravasation in tumor tissues can take place due to the enhanced permeability and retention (EPR) effect. The EPR effect is derived from both a defective structure in blood vessels irrigating tumor tissue, which makes them more permeable to macromolecules and nanoparticles, and collapsed lymphatic vessels, which prevent nanoparticle withdrawal from tumor tissue after extravasation.[81] Even though the EPR effect has been the main rationale behind nanoparticle use in oncology, its broad applicability in humans has been questioned.[82] Furthermore, even if tumor blood vessels might be suitable for nanoparticle extravasation, the high interstitial pressure in solid tumors acts as a barrier for effective extravasation. Generating a certain degree of cavitation (stable or inertial) could provide an additional force to extravasate the nanoparticles instead of relying merely on passive diffusion,[34] and it could also enhance the penetration depth of the nanoparticles in tumor tissue.[83] This concept has been successfully

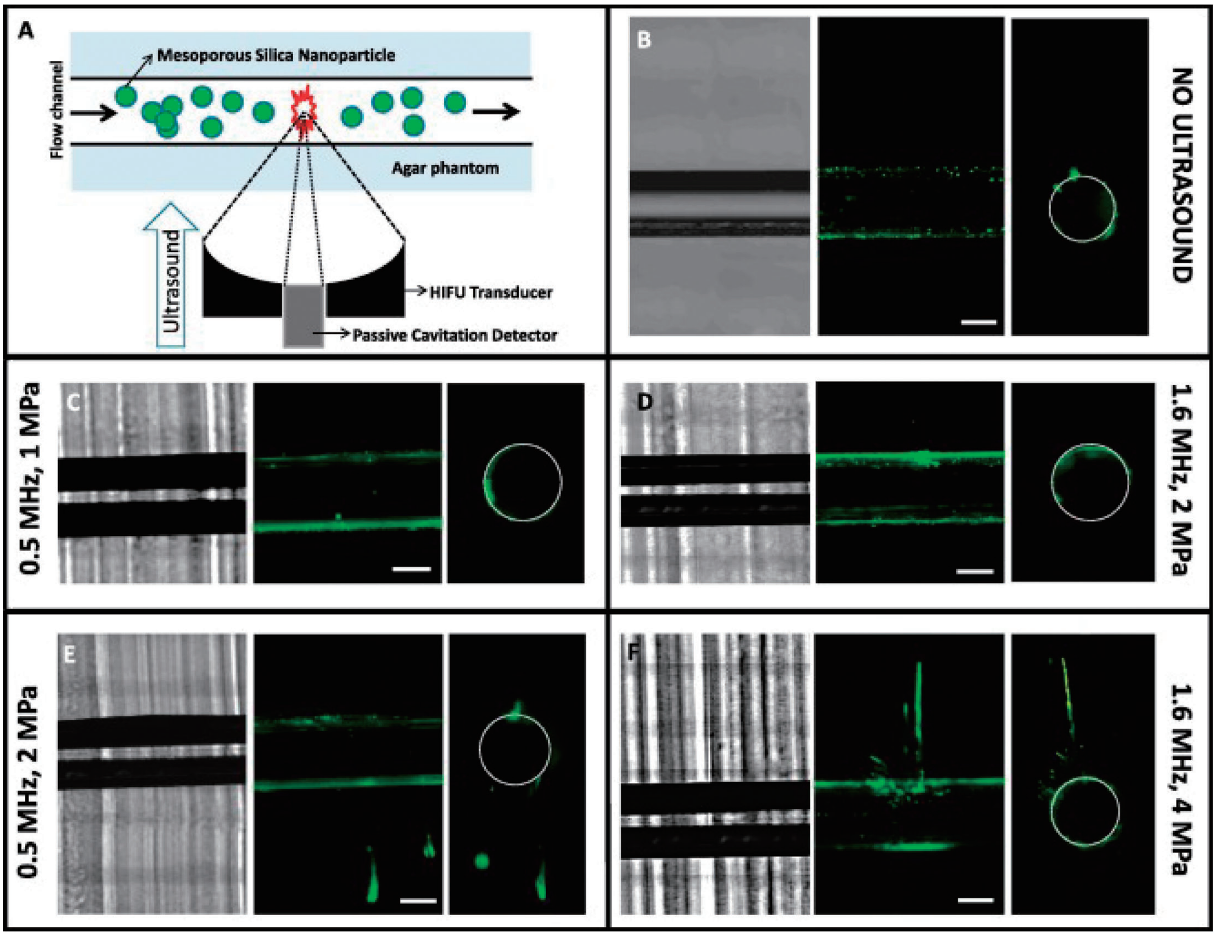

Figure 4. In vitro mesoporous silica nanoparticle delivery in an agarose gel model at different US exposure conditions. Reprinted with permission from ref 89. Copyright 2018 Elsevier.

developed to various degrees in *in vitro* or *in vivo* models with viruses,[84] liposomes,[85] polymeric nanoparticles,[86,87] gold nanoparticles[88] and mesoporous silica nanoparticles[89] (Figure 4).

In order to achieve the level of acoustic cavitation that is necessary to enable nanoparticle extravasation without damaging the surrounding healthy tissues, different cavitation nuclei can be employed to decrease the necessary pressure threshold. Recently, Mannaris *et al.* compared the effect at various frequencies of different cavitation nuclei in this context: microbubbles, nanodroplets and polymer gas-stabilizing nanoparticles.[90] The authors found that deeper and more directional extravasation took place at higher frequencies, and that polymeric gas-trapping produced the most extensive extravasation for the same applied energy.[90] In all these works, a mixture of the therapeutic nanoparticle and the cavitation nuclei has to be administered in order to provide both the cavitation-generation capacity and the desired therapeutic effect. However, recent investigations by different groups have also begun exploring combining both components in a single structure. In that way, Su *et al.* have recently described similar polymeric gas-stabilizing microstructures with multiple gas-stabilizing cavities per particles.[91] In this case, the nanoparticles were made of poly(lactic-co-glycolic acid) and included within the polymeric network a rhodamine dye as a model for small molecule drugs. After showing their potential for inducing their remote implantation by application of HIFU, the authors also confirmed the sustained release of the loaded fluorophore over time.[91] Other recent examples showing combination of the cavitation nuclei and therapeutic nanoparticle functions can be found in the work of Papa *et al.* who employed nanoparticle aggregates that trapped gas within the aggregate and could be dispersed into individual particles while generating cavitation,[53] and in the work by Lv *et al.* who included mesoporous silica nanoparticles within microbubbles for this same purpose.[92]

A limiting factor for the treatment of pathologies of the central nervous system (CNS) is the blood-brain barrier (BBB). The BBB is a restrictive barrier that prevents most toxic (and therapeutic) agents from reaching the CNS.[93] The physical structure of the BBB relies on tight junctions present between specialized endothelial cells. This structure can be reversibly impaired by the generation of focused acoustic cavitation in the region, temporarily enabling the delivery of certain therapeutics to the CNS. The translation of this type of strategy to the clinic could enable using a wide variety of therapeutic agents for different CNS pathologies which might not be suitable today due to biodistribution limitations. So far, different preclinical studies have shown the possibility of using US to enable delivery to the CNS of different nanotherapeutic agents, including nanoparticles for gene therapy,[94,95] magnetic nanoparticles,[93,96] gold nanoparticles,[97–99] polymeric nanoparticles[100] and liposomes.[101] It is worth mentioning that, as was also the case in the previous section, in order to decrease the pressure threshold needed to trigger cavitation up to a safe level for surrounding tissues, different cavitation nuclei must be used to enable successful reversible opening of the BBB for nanoparticle delivery. The most widely used agents for this purpose are microbubbles, which can be co-injected with the therapeutic nanoagent[98,101,102] or complexed directly with them, having both components (therapeutic agent and cavitation nuclei) in a single structure.[103] Some work employing other cavitation nuclei has also been reported, such as using nanobubbles in the context of SNC gene therapy.[95]

Finally, once the nanoparticles are located in the desired tissue or organ, it might be the case that, in order to maximize the therapeutic effect, nanoparticle uptake by the target cells is necessary. US can also be used here to trigger nanoparticle uptake by nearby cells, through different mechanisms. For example, the direct effect of US on the cell membranes can be used to ease nanoparticle introduction within the cell cytoplasm, in a process usually called sonoporation.[104,105] Another option to achieve a similar goal would be developing US-responsive hierarchical targeting strategies. In hierarchical nanoparticle targeting, an uptake-inducing moiety is exposed on the nanoparticle surface after a certain environmental condition is present. Following this line of work, we have recently reported the possibility of employing positively-charged nanoparticles that have been PEGylated through a thermosensitive linker.[106] The presence of PEG chains on the nanoparticle surface provide stealth capabilities and physically hide the positively-charged amino groups on the nanoparticle surface. Upon US exposure, localized heating produces the de-PEGylation of the nanoparticles, exposing their positive surface charge and inducing nanoparticle uptake due to electrostatic interaction of the nanoparticles with the cell membrane. Even though for now this strategy has only been presented as a proof-of-concept, further developments in this direction could be very promising for novel therapeutics.

5. Developing Theranostic Agents

US-based imaging is widely used in clinical practice, and echogenic contrast agents (such as microbubbles) can be employed to improve its performance.[51] By combining these imaging capacities with therapeutic carriers, theranostic agents can be developed. This allows, for example, ensuring the correct injection of an US-triggerable (and echogenic) anesthetic micron-sized liposomal formulation around the desired nerve (Figure 5).[51]

Different types of formulations have been developed as therapeutic agents that simultaneously act as US contrast agents, such as phase-change nanodroplets,[107–110] silica nanoparticles[111] and multilayer capsules.[112] In this way, US imaging can be used to follow nanoparticle accumulation in the target area (for example, by EPR-driven tumor accumulation) and also to trigger drug release when sufficient amount of the therapeutic nanoagent has been detected in the region to treat.[109] US Contrast agents can not only be developed in combination with drug-delivery carriers, but also with agents employed for some of the other therapeutic modalities that we have already discussed here, such as generation of inertial cavitation and SDT.

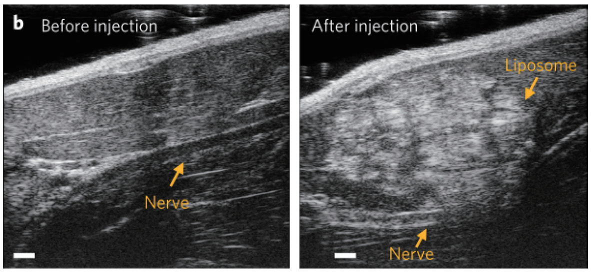

Figure 5. Sonograms before and after US-guided perisciatic injections of a liposomal formulation for anesthesia application. Reprinted with permission from ref 51. Copyright 2017 Springer Nature.

As an example of this, Feng *et al.*[113] developed pH/US dual-responsive gas-generating nanoparticles carrying a sonosensitizer to combine in one single nanostructure US imaging, generation of inertial cavitation and SDT capacities.

6. Conclusion

The development of ultrasound-activated nanomaterials for biomedical application is an extremely active field of research that holds great promise for creating future therapeutic strategies. Among the main examples discussed here, ultrasound has not only been employed to trigger drug release from nanoparticles, but also to produce direct biological effects in combination with nanostructures, to modify nanoparticle biodistribution and to develop theranostic agents. While some work has already been done in combining those strategies, future work will surely delve deeper into strategies that employ several or all of these effects simultaneously. From these combined approaches remarkable synergies can appear, such as employing the feedback from theranostic systems to select the optimal time to trigger drug release, or even to further exploit the effect of triggered drug release by combining it with direct biological effects produced by the US stimulus. Further improvements can also be achieved by combining ultrasound-responsive materials with other stimuli-responsive nanostructures, both for therapeutic and imaging purposes.[114,115] In this way, the advantages of different stimuli can be seized to achieve their combined full potential. Finally, we believe that through the development of all of the recent advances mentioned here, the field of ultrasound-responsive nanomaterials has reached a level of maturity that should now enable these technologies to become closer to the clinical setting. In order to reach the clinic, successful nanostructures should have a simple, reproducible and scalable production process, what can constitute a significant challenge for many of the chemically complex nanosystems here presented. On the other hand, the widespread use of diagnostic ultrasound in hospitals as well as therapeutic ultrasound in physiotherapy and other applications could also accelerate the adoption of these strategies compared to the use of other external stimuli.

This paper was funded by the European Research Council (Grant VERDI; ERC-2015 AdG no. 694160). JL Paris is supported by a post-doctoral fellowship from the Ramón Areces Foundation.

References

1 C. I. C. Crucho, *ChemMedChem* **2015**, *10*, 24.
2 S. Gao, G. Tang, D. Hua, R. Xiong, J. Han, S. Jiang, Q. Zhang, C. Huang, *J. Mater. Chem. B* **2019**, *7*, 709.
3 M. Gisbert-Garzaran, D. Lozano, M. Vallet-Regí, M. Manzano, *RSC Adv.* **2017**, *7*, 132.
4 J. Zhang, J. Li, Z. Shi, Y. Yang, X. Xie, S. M. Lee, Y. Wang, K. W. Leong, M. Chen, *Acta Biomater.* **2017**, *58*, 349.
5 Z.-Y. Li, J.-J. Hu, Q. Xu, S. Chen, H.-Z. Jia, Y.-X. Sun, R.-X. Zhuo, X.-Z. Zhang, *J. Mater. Chem. B* **2015**, *3*, 39.
6 Y. K. Lin, Y. C. Yu, S. W. Wang, R. S. Lee, *RSC Adv.* **2017**, *7*, 43212.
7 A. Popat, B. P. Ross, J. Liu, S. Jambhrunkar, F. Kleitz, S. Z. Qiao, *Angew. Chem., Int. Ed.* **2012**, *51*, 12486.
8 B. P. Timko, T. Dvir, D. S. Kohane, *Adv. Mater.* **2010**, *22*, 4925.
9 M. Martínez-Carmona, D. Lozano, A. Baeza, M. Colilla, M. Vallet-Regí, *Nanoscale* **2017**, *9*, 15967.
10 M. Martínez-Carmona, A. Baeza, M. A. Rodriguez-Milla, J. García-Castro, M. Vallet-Regí, *J. Mater. Chem. B* **2015**, *3*, 5746.
11 J. Lai, X. Mu, Y. Xu, X. Wu, C. Wu, C. Li, J. Chen, Y. Zhao, *Chem. Commun.* **2010**, *46*, 7370.
12 A. Baeza, E. Guisasola, E. Ruiz-Hernández, M. Vallet-Regí, *Chem. Mater.* **2012**, *24*, 517.
13 C. Montis, B. Castroflorio, M. Mendozza, A. Salvatore, D. Berti, P. Baglioni, *J. Colloid Interface Sci.* **2015**, *449*, 317.
14 E. Guisasola, A. Baeza, M. Talelli, D. Arcos, M. Moros, J. M. de la Fuente, M. Vallet-Regí, *Langmuir* **2015**, *31*, 12777.
15 Y. Sheng, E. Beguin, H. Nesbitt, S. Kamila, J. Owen, L. C. Barnsley, B. Callan, C. O'Kane, N. Nomikou, R. Hamoudi, M. A. Taylor, M. Love, P. Kelly, D. O'Rourke, E. Stride, A. P. McHale, J. F. Callan, *J. Controlled Release* **2017**, *262*, 192.
16 A. Schroeder, J. Kost, Y. Barenholz, *Chem. Phys. Lipids* **2009**, *162*, 1.
17 P. Tsaklis, *Human Mov.* **2010**, *11*, 91.
18 K. R. Rohr, J. A. Rooney, *Biophys. J.* **1978**, *23*, 33.
19 D. O. Draper, J. C. Castel, D. Castel, *J. Orthop. Sports Phys. Ther.* **1995**, *22*, 142.
20 X. B. Wang, Q. H. Liu, P. Wang, W. Tang, Q. Hao, *Ultrasonics* **2008**, *48*, 135.
21 R. Carlisle, J. Choi, M. Bazan-Peregrino, R. Laga, V. Subr, L. Kostka, K. Ulbrich, C.-C. Coussios, L. W. Seymour, *JNCI, J. Natl. Cancer Inst.* **2013**, *105*, 1701.
22 A. K. W. Wood, C. M. Sehgal, *Ultrasound Med. Biol.* **2015**, *41*, 905.
23 R. J. E. van den Bijgaart, D. C. Eikelenboom, M. Hoogenboom, J. J. Fütterer, M. H. den Brok, G. J. Adema, *Cancer Immunol. Immunother.* **2017**, *66*, 247.
24 K. Hynynen, N. McDannold, N. A. Sheikov, F. A. Jolesz, N. Vykhodtseva, *Neuroimage* **2005**, *24*, 12.
25 B. Theek, M. Baues, T. Ojha, D. Möckel, S. K. Veettil, J. Steitz, L. van Bloois, G. Storm, F. Kiessling, T. Lammers, *J. Controlled Release* **2016**, *231*, 77.
26 J. Unga, M. Hashida, *Adv. Drug Delivery Rev.* **2014**, *72*, 144.
27 A. Fenster, D. B. Downey, H. N. Cardinal, *Phys. Med. Biol.* **2001**, *46*, R67.
28 J. E. Kennedy, G. R. ter Haar, D. Cranston, *Br. J. Radiol.* **2003**, *76*, 590.
29 H. Grüll, S. Langereis, *J. Controlled Release* **2012**, *161*, 317.
30 J. Kobayashi, T. Okano, *Bull. Chem. Soc. Jpn.* **2019**, *92*, 817.
31 I. Rosenthal, J. Z. Sostaric, P. Riesz, *Ultrason. Sonochem.* **2004**, *11*, 349.
32 S. R. Sirsi, M. A. Borden, *Adv. Drug Delivery Rev.* **2014**, *72*, 3.
33 B. E. O'Neill, H. Vo, M. Angstadt, K. P. C. Li, T. Quinn, V. Frenkel, *Ultrasound Med. Biol.* **2009**, *35*, 416.
34 C. D. Arvanitis, M. Bazan-Peregrino, B. Rifai, L. W. Seymour, C. C. Coussios, *Ultrasound Med. Biol.* **2011**, *37*, 1838.
35 M. Kuroki, K. Hachimine, H. Abe, H. Shibaguchi, M. Kuroki, S. Maekawa, J. Yanagisawa, T. Kinugasa, T. Tanaka, Y. Yamashita, *Anticancer Res.* **2007**, *27*, 3673.
36 A. Yildirim, R. Chattaraj, N. T. Blum, A. P. Goodwin, *Chem. Mater.* **2016**, *28*, 5962.
37 J. Turánek, A. D. Miller, Z. Kauerová, R. Lukáč, J. Mašek, Š. Koudelka, M. Raška, in *Advances in Bioengineering*, InTech, **2015**.
38 J. Y. Lee, D. Carugo, C. Crake, J. Owen, M. de Saint Victor, A. Seth, C. Coussios, E. Stride, *Adv. Mater.* **2015**, *27*, 5484.
39 J. J. Kwan, S. Graham, R. Myers, R. Carlisle, E. Stride, C. C. Coussios, *Phys. Rev. E: Stat., Nonlin., Soft Matter Phys.* **2015**, *92*, 023019.
40 J. J. Kwan, R. Myers, C. M. Coviello, S. M. Graham, A. R. Shah, E. Stride, R. C. Carlisle, C. C. Coussios, *Small* **2015**, *11*, 5305.
41 P. Cintas, S. Tagliapietra, M. Caporaso, S. Tabasso, G. Cravotto, *Ultrason. Sonochem.* **2015**, *25*, 8.
42 F. Li, C. Xie, Z. Cheng, H. Xia, *Ultrason. Sonochem.* **2016**, *30*, 9.
43 Y. Li, P. Wang, P. Zhao, S. Zhu, X. Wang, Q. Liu, *Ultrasonics* **2012**, *52*, 490.
44 Q. Liu, X. Wang, P. Wang, H. Qi, K. Zhang, L. Xiao, *Ultrasonics* **2006**, *45*, 56.
45 Z. J. Dai, S. Li, J. Gao, X. N. Xu, W. F. Lu, S. Lin, X. J. Wang, *Med. Hypotheses* **2013**, *80*, 300.
46 T. G. McKenzie, E. Colombo, Q. Fu, M. Ashokkumar, G. G. Qiao, *Angew. Chem., Int. Ed.* **2017**, *56*, 12302.
47 M. J. Ernsting, A. Worthington, J. P. May, T. Tagami, M. C. Kolios, S.-D. Li, 2011 IEEE International Ultrasonics Symposium, 1–4.
48 B. Geers, I. Lentacker, N. N. Sanders, J. Demeester, S. Meairs, S. C. De Smedt, *J. Controlled Release* **2011**, *152*, 249.
49 J. Wang, M. Pelletier, H. Zhang, H. Xia, Y. Zhao, *Langmuir* **2009**, *25*, 13201.
50 A. Schroeder, R. Honen, K. Turjeman, A. Gabizon, J. Kost, Y. Barenholz, *J. Controlled Release* **2009**, *137*, 63.
51 A. Y. Rwei, J. L. Paris, B. Wang, W. Wang, C. D. Axon, M. Vallet-Regí, R. Langer, D. S. Kohane, *Nat. Biomed. Eng.* **2017**, *1*, 644.
52 J. Xuan, O. Boissière, Y. Zhao, B. Yan, L. Tremblay, S. Lacelle, H. Xia, Y. Zhao, *Langmuir* **2012**, *28*, 16463.
53 A. L. Papa, N. Korin, M. Kanapathipillai, A. Mammoto, T. Mammoto, A. Jiang, R. Mannix, O. Uzun, C. Johnson, D. Bhatta, G. Cuneo, D. E. Ingber, *Biomaterials* **2017**, *139*, 187.
54 J. L. Paris, M. V. Cabañas, M. Manzano, M. Vallet-Regí, *ACS Nano* **2015**, *9*, 11023.
55 J. L. Paris, G. Villaverde, M. V. Cabañas, M. Manzano, M. Vallet-Regí, *J. Mater. Chem. B* **2018**, *6*, 2785.
56 J. L. Paris, P. de la Torre, M. V. Cabañas, M. Manzano, M. Grau, A. I. Flores, M. Vallet-Regí, *Nanoscale* **2017**, *9*, 5528.
57 C. Prasad, R. Banerjee, *J. Pharmacol. Exp. Ther.* **2019**, *370*,

876.
58 C. H. Wu, M. K. Sun, J. Shieh, C. S. Chen, C. W. Huang, C. A. Dai, S. W. Chang, W. S. Chen, T. H. Young, *Ultrasonics* **2018**, *83*, 157.
59 P. C. Lyon, M. D. Gray, C. Mannaris, L. K. Folkes, M. Stratford, L. Campo, D. Y. F. Chung, S. Scott, M. Anderson, R. Goldin, R. Carlisle, F. Wu, M. R. Middleton, F. V. Gleeson, C. C. Coussios, *Lancet Oncol.* **2018**, *19*, 1027.
60 M. Vallet-Regí, A. Rámila, R. P. del Real, J. Pérez-Pariente, *Chem. Mater.* **2001**, *13*, 308.
61 J. L. Paris, P. de la Torre, M. V. Cabañas, M. Manzano, A. I. Flores, M. Vallet-Regí, *Acta Biomater.* **2019**, *83*, 372.
62 S. Lee, L. Al-Kaabi, A. Mawart, A. Khandoker, H. Alsafar, H. F. Jelinek, K. Khalaf, J. H. Park, Y. C. Kim, *J. Drug Targeting* **2018**, *26*, 172.
63 Y. Xin, Q. Qi, Z. Mao, X. Zhan, *Int. J. Pharm.* **2017**, *528*, 47.
64 R. D. Airan, R. A. Meyer, N. P. K. Ellens, K. R. Rhodes, K. Farahani, M. G. Pomper, S. D. Kadam, J. J. Green, *Nano Lett.* **2017**, *17*, 652.
65 B. Zhu, L. Wang, J. Huang, X. Xiang, Y. Tang, C. Cheng, F. Yan, L. Ma, L. Qiu, *J. Mater. Chem. B* **2019**, *7*, 4581.
66 X. Gao, P. Zhu, L. Yu, L. Yang, Y. Chen, *Adv. Healthc. Mater.* **2019**, *8*, 1801350.
67 Y. Zhong, Y. Zhang, J. Xu, J. Zhou, J. Liu, M. Ye, L. Zhang, B. Qiao, Z. G. Wang, H. T. Ran, D. Guo, *ACS Nano* **2019**, *13*, 3387.
68 M. de Saint Victor, L. C. Barnsley, D. Carugo, J. Owen, C. C. Coussios, E. Stride, *Ultrasound Med. Biol.* **2019**, *45*, 1151.
69 A. Ishijima, S. Yamaguchi, T. Azuma, E. Kobayashi, Y. Shibasaki, T. Nagamune, I. Sakuma, *Cancer Rep.* **2019**, *2*, 1.
70 P. Huang, X. Qian, Y. Chen, L. Yu, H. Lin, L. Wang, Y. Zhu, J. Shi, *J. Am. Chem. Soc.* **2017**, *139*, 1275.
71 A. Shanei, A. Sazgarnia, N. T. Meibodi, H. Eshghi, H. Mohammad-Khayyat, H. Esmaily, N. A. Kakhki, *Iran. J. Basic Med. Sci.* **2012**, *15*, 759.
72 C. McEwan, J. Owen, E. Stride, C. Fowley, H. Nesbitt, D. Cochrane, C. C. Coussios, M. Borden, N. Nomikou, A. P. McHale, J. F. Callan, *J. Controlled Release* **2015**, *203*, 51.
73 J. Chen, H. Luo, Y. Liu, W. Zhang, H. Li, T. Luo, K. Zhang, Y. Zhao, J. Liu, *ACS Nano* **2017**, *11*, 12849.
74 K. Logan, F. Foglietta, H. Nesbitt, Y. Sheng, T. McKaig, S. Kamila, J. Gao, N. Nomikou, B. Callan, A. P. McHale, J. F. Callan, *Eur. J. Pharm. Biopharm.* **2019**, *139*, 224.
75 Z. Li, J. Han, L. Yu, X. Qian, H. Xing, H. Lin, M. Wu, T. Yang, Y. Chen, *Adv. Funct. Mater.* **2018**, *28*, 1800145.
76 N. Nomikou, K. Curtis, C. McEwan, B. M. G. O'Hagan, B. Callan, J. F. Callan, A. P. McHale, *Acta Biomater.* **2017**, *49*, 414.
77 X. Pang, Q. Xiao, Y. Cheng, E. Ren, L. Lian, Y. Zhang, H. Gao, X. Wang, W. Leung, X. Chen, G. Liu, C. Xu, *ACS Nano* **2019**, *13*, 2427.
78 R. F. V. Lopez, J. E. Seto, D. Blankschtein, R. Langer, *Biomaterials* **2011**, *32*, 933.
79 D. Huang, M. Sun, Y. Bu, F. Luo, C. Lin, Z. Lin, Z. Weng, F. Yang, D. Wu, *J. Mater. Chem. B* **2019**, *7*, 2330.
80 H.-L. Wang, P.-F. Fan, X.-S. Guo, J. Tu, Y. Ma, D. Zhang, *Chin. Phys. B* **2016**, *25*, 124314.
81 H. Maeda, H. Nakamura, J. Fang, *Adv. Drug Delivery Rev.* **2013**, *65*, 71.
82 J. W. Nichols, Y. H. Bae, *J. Controlled Release* **2014**, *190*, 451.
83 S. J. Grainger, J. V. Serna, S. Sunny, Y. Zhou, C. X. Deng, M. E. H. El-Sayed, *Mol. Pharm.* **2010**, *7*, 2006.
84 M. Bazan-Peregrino, C. D. Arvanitis, B. Rifai, L. W. Seymour, C.-C. Coussios, *J. Controlled Release* **2012**, *157*, 235.
85 E. Thomas, J. U. Menon, J. Owen, I. Skaripa-Koukelli, S. Wallington, M. Gray, C. Mannaris, V. Kersemans, D. Allen, P. Kinchesh, S. Smart, R. Carlisle, K. A. Vallis, *Theranostics* **2019**, *9*, 5595.
86 S. Eggen, S.-M. Fagerland, Ý. Mørch, R. Hansen, K. Søvik, S. Berg, H. Furu, A. D. Bøhn, M. B. Lilledahl, A. Angelsen, B. Angelsen, C. de Lange Davies, *J. Controlled Release* **2014**, *187*, 39.
87 M. Figueiredo, R. Esenaliev, *J. Drug Deliv.* **2012**, 767839.
88 C. Mannaris, B. M. Teo, A. Seth, L. Bau, C. Coussios, E. Stride, *Adv. Healthc. Mater.* **2018**, *7*, 1800184.
89 J. L. Paris, C. Mannaris, M. V. Cabañas, R. Carlisle, M. Manzano, M. Vallet-Regí, C. C. Coussios, *Chem. Eng. J.* **2018**, *340*, 2.
90 C. Mannaris, L. Bau, M. Grundy, M. Gray, H. Lea-Banks, A. Seth, B. Teo, R. Carlisle, E. Stride, C. C. Coussios, *Ultrasound Med. Biol.* **2019**, *45*, 954.
91 X. Su, R. G. Thomas, L. D. Bharatula, J. J. Kwan, *Sci. Rep.* **2019**, *9*, 1.
92 Y. Lv, Y. Cao, P. Li, J. Liu, H. Chen, W. Hu, L. Zhang, *Adv. Healthc. Mater.* **2017**, *6*, 1700354.
93 H.-L. Liu, P.-Y. Chen, H.-W. Yang, J.-S. Wu, I.-C. Tseng, Y.-J. Ma, C.-Y. Huang, H.-C. Tsai, S.-M. Chen, Y.-J. Lu, C.-Y. Huang, M.-Y. Hua, Y.-H. Ma, T.-C. Yen, K.-C. Wei, *J. Magn. Reson. Imaging* **2011**, *34*, 1313.
94 B. P. Mead, P. Mastorakos, J. S. Suk, A. L. Klibanov, J. Hanes, R. J. Price, *J. Controlled Release* **2016**, *223*, 109.
95 K. Ogawa, Y. Fuchigami, M. Hagimori, S. Fumoto, K. Maruyama, S. Kawakami, *Eur. J. Pharm. Biopharm.* **2019**, *137*, 1.
96 H.-L. Liu, M.-Y. Hua, H.-W. Yang, C.-Y. Huang, P.-C. Chu, J.-S. Wu, I.-C. Tseng, J.-J. Wang, T.-C. Yen, P.-Y. Chen, K.-C. Wei, *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 15205.
97 A. B. Etame, R. J. Diaz, M. A. O'Reilly, C. A. Smith, T. G. Mainprize, K. Hynynen, J. T. Rutka, *Nanomedicine* **2012**, *8*, 1133.
98 R. J. Diaz, P. Z. McVeigh, M. A. O'Reilly, K. Burrell, M. Bebenek, C. Smith, A. B. Etame, G. Zadeh, K. Hynynen, B. C. Wilson, J. T. Rutka, *Nanomedicine* **2014**, *10*, e1075.
99 T. G. Chan, S. V. Morse, M. J. Copping, J. J. Choi, R. Vilar, *ChemMedChem* **2018**, *13*, 1311.
100 L. Yao, Q. Song, W. Bai, J. Zhang, D. Miao, M. Jiang, Y. Wang, Z. Shen, Q. Hu, X. Gu, M. Huang, G. Zheng, X. Gao, B. Hu, J. Chen, H. Chen, *Biomaterials* **2014**, *35*, 3384.
101 M. Aryal, N. Vykhodtseva, Y.-Z. Zhang, J. Park, N. McDannold, *J. Controlled Release* **2013**, *169*, 103.
102 E. Nance, K. Timbie, G. W. Miller, J. Song, C. Louttit, A. L. Klibanov, T.-Y. Shih, G. Swaminathan, R. J. Tamargo, G. F. Woodworth, J. Hanes, R. J. Price, *J. Controlled Release* **2014**, *189*, 123.
103 S.-W. Ha, K. Hwang, J. Jin, A.-S. Cho, T. Y. Kim, S. Il Hwang, H. J. Lee, C.-Y. Kim, *Int. J. Nanomed.* **2019**, *14*, 3743.
104 Y.-X. J. Wang, K. C.-F. Leung, W.-H. Cheung, H.-H. Wang, L. Shi, D.-F. Wang, L. Qin, A. T. Ahuja, *J. Magn. Reson. Imaging* **2010**, *31*, 1508.
105 I. De Cock, G. Lajoinie, M. Versluis, S. C. De Smedt, I. Lentacker, *Biomaterials* **2016**, *83*, 294.
106 J. L. Paris, M. Manzano, M. V. Cabañas, M. Vallet-Regí, *Nanoscale* **2018**, *10*, 6402.
107 F. Baghbani, M. Chegeni, F. Moztarzadeh, J. A. Mohandesi, M. Mokhtari-Dizaji, *Mater. Sci. Eng., C* **2017**, *77*, 698.

108 F. Baghbani, F. Moztarzadeh, *Colloids Surf., B* **2017**, *153*, 132.
109 Y. Toumia, B. Cerroni, F. Domenici, H. Lange, L. Bianchi, M. Cociorb, F. Brasili, E. Chiessi, E. D'Agostino, K. Van Den Abeele, S. V. Heymans, J. D'Hooge, G. Paradossi, *Langmuir* **2019**, *35*, 10116.
110 D. Meng, L. Guo, D. Shi, X. Sun, M. Shang, X. Zhou, J. Li, *Nanomedicine* **2019**, *19*, 2549.
111 N. T. Blum, A. Yildirim, C. Gyorkos, D. Shi, A. Cai, R. Chattaraj, A. P. Goodwin, *ACS Appl. Mater. Interfaces* **2019**, *11*, 15233.
112 J. Chen, S. Ratnayaka, A. Alford, V. Kozlovskaya, F. Liu, B. Xue, K. Hoyt, E. Kharlampieva, *ACS Nano* **2017**, *11*, 3135.
113 Q. Feng, W. Zhang, X. Yang, Y. Li, Y. Hao, H. Zhang, L. Hou, Z. Zhang, *Adv. Healthc. Mater.* **2018**, *7*, 1700957.
114 S. Eom, G. Choi, H. Nakamura, J.-H. Choy, *Bull. Chem. Soc. Jpn.* **2020**, *93*, 1.
115 K. Bhattacharyya, S. Mukherjee, *Bull. Chem. Soc. Jpn.* **2018**, *91*, 447.

