Abstract
The integration of therapeutic interventions with diagnostic imaging has been recognized as one of the next technological developments that will have a major impact on medical treatments. Therapeutic applications using ultrasound, for example thermal ablation, hyperthermia or ultrasound induced drug delivery, are examples for image-guided interventions that are currently investigated. While thermal ablation using MR-HIFU is entering the clinic, ultrasound mediated drug delivery is still in a research phase, but holds promise to enable new applications in localized treatments. The use of ultrasound for the delivery of drugs has been demonstrated in particular the field of cardiology and oncology for a variety of therapeutics ranging from small drug molecules to biologics and nucleic acids exploiting temperature or pressure mediated delivery schemes.

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Key Terms

<table>
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<th><strong>(Focused) Ultrasound</strong>: Ultrasound is cyclic pressure wave with a frequency higher than the upper limit of human hearing. Ultrasound finds widespread application in medicine, for example in diagnostic imaging and therapeutic applications. Typical ultrasound frequencies for diagnostic imaging are in the range of 1-18 MHz. If focused on a spot inside the tissue, energy dissipation causes local heating of the tissue that can be used for thermal ablation of pathogenic tissues. Ablation by focused ultrasound is a technology which can be used in noninvasive medical treatment of several conditions, including uterine fibroids and different cancers. [1].</th>
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<td><strong>Microbubbles</strong>: Microbubbles are used in medical diagnostics as a contrast agent for ultrasound imaging. These microbubbles are intravenously administered and consist out of gas bubbles typically having diameters in the range 1-5 micrometers, and are stabilized with a layer to prevent rapid dissolution in the blood [2].</td>
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<td><strong>Gene therapy</strong>: Gene therapy is defined as the process of modifying the genetic content of a cell and/or tissue resulting in overexpression or (partly) inhibition of a certain protein. These modifications can either be genomic (direct integration in the genomic DNA) for permanent genetic modification or episomal (non-integrating) resulting in mostly transient modifications and therefore transient therapeutic effects. Genetic modifications can be accomplished by introducing genes, for example via plasmid DNA</td>
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(pDNA) or via a viral transfection route or further downstream by RNA interference (RNAi). RNAi is a system within living cells for controlling which genes are active and how active they are. These small interfering RNAs (siRNA) are the direct products of genes that can bind to specific other RNAs and either increase or decrease their activity, for example by preventing a messenger RNA from producing a protein.

**Nanomedicine:** Nanomedicine is the application of nanotechnology in health care. It involves complex materials with dimensions ranging from nanometers up to microns, which can be manufactured in a controlled way through the exploration of specific interactions between self-assembling molecules for bottom-up creation of nanostructures. With sizes matching those in nature, interactions between man-made and biological material can be fine tuned to a high degree leading to new applications in medicine.

**Magnetic resonance imaging:** Magnetic Resonance Imaging (MRI, or magnetic resonance tomography, MRT) is a medical imaging technique used in radiology. MRI exploits the different amount of water protons, and – more importantly – the different magnetic properties of these protons in different tissues to generate an image. For soft tissues, MRI provides superb images with (sub) millimeter resolution. The different magnetic properties of water can be modulated by contrast agents (CAs) for MRI. The measure of how effective a CA changes these magnetic properties of water in the direct environment is denoted *relaxivity* (*r*, \( \text{mM}^{-1}\text{s}^{-1} \)).

**Image-guided intervention:** More and more therapeutic interventions are performed under guidance of one of the diagnostic imaging modalities such as x-ray/CT, ultrasound or MRI. Image guidance is required to navigate interventional tools such as catheters, needles, heat sources, or external beam radiotherapy to the correct location in the body and ensure that the therapy is applied to the pathogenic tissue. Examples of image guided interventions include procedures where catheterization is required, such as stent placement or radiotherapy. Besides spatial feedback, real time temperature mapping is crucial in all thermal therapies. Examples are catheter delivered radio-frequency ablation, cryotherapy, of HIFU ablation, where a temperature feedback is
essential for safety and efficacy in addition to the spatial guidance.

**Temperature sensitive liposome:** Liposomes are spherical vesicles consisting of an aqueous core (lumen) enclosed by a bilayer of phospholipids. Liposomes can be prepared with diameters between 50 nm up to microns by size extrusion techniques, though for medical *in-vivo* applications small diameters are preferred. For application as drug delivery vehicles, hydrophilic drugs can be entrapped in the lumen, or hydrophobic drugs can be inserted into the lipid membrane. Currently, several liposomal drug formulations of small molecular cytotoxic compounds are approved for oncology applications, such as Doxil®/Caelyx®, or Myocet®. Upon injection, these drug loaded liposomes accumulate in tumor tissue due to the enhanced permeability and retention effect (EPR). Temperature sensitive liposomes are formed by lipids that exhibit a gel to sol transition at temperatures a few degrees above the physiological temperature (40-43°C), a range easily obtainable by local hyperthermia. When the transition temperature is reached the liposomal bilayer becomes leaky leading to rapid drug release [3, 4].

**Thermal ablation and hyperthermia:** Pathogenic tissue can be heated to temperatures that induce denaturing of proteins followed by necrosis of the tissue. The temperature dose that is necessary to induce necrosis is given by the integral under the temperature-time curve and is expressed in equivalent minutes. While it takes at 43°C 240 minutes to induce cell death, tissue necrosis is almost instant when heated to 60°C. Thermal ablation therefore operates at temperatures around 60°C and above to ensure deposition of lethal temperature dose within a practical time window [5-7]. In hyperthermia the body is regionally or locally warmed to temperatures between 40-43°C, where the heat itself doesn’t have a direct therapeutic effect. Instead, hyperthermia induces synergistic effects in systemic chemotherapy or radiation treatment as it makes tumors, for various reasons, more sensitive to the effects of radiation and certain anti-cancer drugs [8-10].

**Sonoporation and extravasation:** Sonoporation denotes the ultrasound-induced transient change of cellular membrane permeability. As a consequence, sonoporation can mediate the cellular uptake of compounds that would otherwise not pass the cell
membrane. This can be exploited for ultrasound mediated drug uptake, such as cell impermeable oligonucleotide based compounds. Sonoporation, although not fully understood, results from the interaction of cells with ultrasound and gas bubbles. Bursting of these microbubbles with ultrasound (cavitation) leads to high pressure jets inducing porosity in cell membranes and permeabilization of the endothelial layer (enhanced extravasation). Cavitation and therefore also sonoporation also occurs at high enough acoustic intensities without the presence of injected microbubbles [11-15].

Introduction

Image-guided interventions

Over the last years, medical imaging modalities such as x-ray, computer tomography (CT) ultrasound (US) or magnetic resonance imaging (MRI) moved gradually from pure diagnostic use into therapeutic applications that benefit from image guidance [16, 17]. In first instance, these are all interventional procedures where pathogenic tissue is treated with the help of a device such as a catheter or where drugs are directly injected into the target tissue.[18] Common examples are stent placements or electrophysiological procedures in the heart that are performed under x-ray imaging for precise spatial guidance. Localization and spatial information are equally crucial for catheter-based arterial embolization procedures e.g. for treatment of uterine fibroids [19]. Also in brachytherapy, where radioactive seeds are placed in the diseased tissue, precise therapy planning and subsequent deposition at a well defined location is crucial[20]. Typically, these procedures are performed under x-ray (fluoroscopy), or US guidance. Besides spatial information, imaging modalities can provide additional readout essential for certain procedures. One example are thermal therapies, where tissue is locally heated (ablation therapy, hyperthermia) or cooled cryo-ablation). One emerging technique is for example ultrasound-induced thermal ablation of tissues, which is now clinically used for treatment of uterine fibroids [21-23] and in a more explorative state for treatment of malignant cancers [5, 7, 22, 24, 25]. In this procedure, a lethal thermal dose needs to be deposited in the target tissue, which requires precise spatial as well as temperature information. Consequently, MRI is now the modality of choice as MRI not only provides a superb soft tissue contrast for
spatial guidance, but has also the ability to acquire in vivo and in real time a temperature map [7, 26-28]. These two readouts serve as a feedback to guide the focused ultrasound beam until all target tissue is ablated.

All non-invasive and minimally invasive interventional procedures involve three distinct phases which can be described as “See, Reach and Treat”. Diagnostic imaging allows to “See” and delineate the pathogenic tissue for planning of the procedure. In the “Reach” phase, real time imaging matches the positioning of interventional devices, for example a catheter, with the morphological information to ensure correct localization. Finally, imaging provides during the “Treat” phase a real time feedback that leads to improved efficacy of the therapeutic intervention, as well as improved safety.

Image-guided drug delivery

While many of above interventional procedures are mainly device oriented, new image guided procedures emerge that also contain a drug compound in the treatment scheme. One already clinically used example is transarterial chemoembolization (TACE) for treatment of liver metastasis, where tumor tissue is locally exposed to a high dose of chemotherapy combined with an embolization step to prevent rapid drug washout from the target tissue[18, 29, 30].

Another approach is to systemically inject drugs or drug loaded carriers but use an exogenous stimulus to activate the drug at the place of interest. Preferably this stimulus can be provided in a minimally invasive or even non-invasive manner with high spatial precision. Over the last decade ultrasound emerged here as a very promising modality for this purpose. Ultrasound is a pressure wave that interacts with tissue in several ways. In first instance, the pressure waves have a mechanical interaction with the tissue. Just like light, ultrasound can also be focused leading to a high intensity in the focal volume, therefore also termed (high intensity) focused ultrasound (HIFU, sometimes also FUS or HIFUS). However, contrary to light, ultrasound waves can penetrate through tissue, which allows targeting deep seated tissue. In the focus spot, intensities can be 3-4 orders of magnitude higher than in the far and near field of the transducer. Interaction with the tissue in the focus spot and energy dissipation provides either a strong
mechanical stimulus, or – depending on the length of time the ultrasound is applied –
leads to local heating. Both pressure and temperature stimuli can be exploited for local
drug delivery applications (Figure 1) [1, 31-33].

For pressure mediated drug delivery, intravenously injected microbubbles, usually used
as ultrasound contrast agents, play a crucial role. These microbubbles typically have
diameters in the range of 1-5 micrometers and efficiently interact with the ultrasound
wave. Oscillation of the microbubble or eventually ultrasound induced cavitation within
the vasculature causes a transient change in permeability of the endothelial layer of
blood vessels as well as cell membranes.[1, 12-15, 34] Application of this effect to
disrupt natural barriers like the blood brain barrier was recently reviewed in this
journal.[35] The increase in permeability can facilitate extravasation of drug compounds
out of the vasculature into the interstitial space as well as uptake of these compounds
into the cell. Naturally, especially drugs that do not have a high free volume of
distribution and/or show limited or no cellular uptake benefit from this approach.
Showing neither extravasation nor cellular uptake by itself, oligonucleotide based drugs
like pDNA or siRNA are the most prominent candidates to benefit from ultrasound
mediated drug delivery [31, 36, 37]. Pressure induced drug delivery using ultrasound
also benefits from image guidance for spatial control. Though several imaging
modalities can be combined with ultrasound, for this application diagnostic ultrasound
may provide additional information as cavitation of microbubbles by therapeutic
ultrasound and subsequent replenishment of the vessels can be followed [38].

The ability to locally heat the tissue can be exploited to trigger drug release from
temperature sensitive drug carriers. This concept was already proposed thirty years ago
by Yatvin and Weinstein using temperature sensitive liposomes that encapsulate a
small molecular weight drug [3, 4, 39]. Injected systemically, the liposomes stably
encapsulate the drug in their aqueous lumen and prevent rapid drug distribution across
all tissues as well as rapid renal excretion. When entering the hyperthermic tissue,
which is warmed to temperatures between 41- 43°C, the temperature sensitive
liposomes rapidly release their drug payload intravascular, leading to a high local drug
concentration in the tissue. Though this concept was introduced 30 years ago, many
issues had to be tackled. First of all, the temperature sensitive drug formulation had to be optimized to ensure stable encapsulation at body temperature and rapid release at elevated temperatures.[39] Secondly, a technique had to be developed to warm up a tissue and keep it at a well defined temperature.[9] Magnetic Resonance guided High Focused (MRgHIFU) may very well be one technology option well suited to address above requirements.[7, 32, 40] The concept to use MRI to provide a real time spatial as well as temperature measurement was introduced in the 90ties.[41, 42] Yet, it took another step of technology development to also provide a closed-loop temperature feedback to the ultrasound system, in order to control the deposition of a well defined thermal dose.[26, 27] In the temperature regime used for thermal ablation, a lethal temperature dose is deposited in the target to induce necrosis. MRgHIFU can also be used to apply hyperthermia in the temperature range of 41-43ºC in combination with temperature sensitive drug delivery systems as has been shown recently.[43-46]

In the following sections, temperature and pressure mediated drug delivery will be reviewed in more detail.

Figure 1

Figure 1: Ultrasound can be employed to non-invasively and locally either raises the temperature inside the body for ablation or to induce hyperthermia. Hyperthermia as such already shows synergistic effects when combined with systemic chemotherapy. Furthermore, hyperthermia can be used to trigger local drug delivery using temperature sensitive drug carriers. Alternatively, the pressure wave associated ultrasound can be used to mediate drug delivery as well, usually in combination with microbubbles.
Mechanisms of ultrasound-mediated drug delivery

Temperature sensitive drug delivery

The new approach “local and temperature-induced drug delivery under image guidance” holds promise to improve the therapeutic window in cancer therapy. This approach is the logical merger of three important developments coming out of clinical, technological and chemical research over the last years that join forces and may offer a new breakthrough solution in minimally invasive treatment of cancer.

Hyperthermia

Regional hyperthermia was shown to act synergistically in combination with systemic chemotherapy and radiotherapy leading to an extended long term survival in patients suffering from soft tissue sarcoma, melanoma, recurrent breast cancer and cervical cancer.[47-52] Three different modes of action are currently attributed to beneficial effects of hyperthermia in combination with chemotherapy, namely direct thermal toxicity to cancer cells, increases drug efficacy at elevated temperatures, and induction of tumoricidal immune responses [8-10, 47]. There is little doubt that enhanced synergistic effects with systemic chemotherapy will show up for these and other types of cancer, though thorough clinical evaluation is still needed.

Liposomes as temperature-sensitive drug carriers

In chemistry, liposomal formulations of small drugs like doxorubicin, e.g. Doxil®/Caelyx® entered the clinic and reduced toxic side effects. However, in some clinical studies improved efficacy was lacking, mainly attributed to reduced bioavailability of the liposomal encapsulated drug. This problem is addressed with new temperature sensitive liposomal formulations that allow local heat triggered release of a drug, leading to a high local concentration of chemotherapeutics inside the target tissue, e.g. tumor. Further development of a temperature sensitive liposomal formulation tailored to this application was needed. New formulations of TSLs comprising lysolipids
and PEGylated lipids in the lipid bilayer provided a drug carrier with rapid release kinetic at hyperthermia (T=42°C) and acceptable circulations times and stability at body temperature.[39, 53] Incorporation of lysolipids into the liposomal membrane presumably facilitates the formation of transient pores in the lipid bilayer around the lipid melting temperature leading to a more rapid and quantitative release of the encapsulated contents. Meanwhile alternative formulations are emerging that avoid the use of lysolipids and PEGylated lipids and may show superior pharmacokinetic properties for above delivery strategy.[54, 55]

Devices for temperature sensitive drug delivery

In technology, devices were developed to provide either local or regional hyperthermia. However, difficult treatment planning and poor control of the tissue temperature in a well defined area is the major drawback for these approaches. Recently, high-focused ultrasound (HIFU) was developed into a clinical technology that can be used to locally warm up tissues within a well-defined target volume. Key is the image guidance by Magnetic Resonance Imaging (MRI) to provide spatial and temporal temperature feedback to the ultrasound control unit. Electronic beam steering using a phased array ultrasound transducer in combination with real time temperature mapping by MRI allows volumetric heating of tumor tissue to a constant temperature over a longer time and deposition of a well defined heat dose.[26, 27] Though MRgHIFU is currently clinically approved for thermal ablation of tissues at temperatures around T=60°C, it is equally well suited for providing local hyperthermia at T=42°C over 30 minutes or longer for local and temperature-triggered drug delivery application, as shown recently.[45]

Examples for temperature-induced drug delivery: liposomal doxorubicin

It is beyond the scope to give a detailed review on temperature induced drug delivery where hyperthermia is induced by other means than ultrasound. With several preclinical studies having successfully demonstrated temperature induced drug delivery and a temperature sensitive formulation in clinical trials, there is much hope that this approach
will lead to a new way of localized cancer treatment. However, most current techniques to induce hyperthermia suffer from limited temperature and spatial control or are of invasive nature. While MRgHIFU holds great promise to address these issues, little work has been published using this technique for local temperature induced drug delivery.

Recently three studies were published that use MRgHIFU with a closed-loop temperature control to release doxorubicin from temperature sensitive liposomes.[43-45] Staruch et al. showed temperature induced drug delivery of doxorubicin in thigh muscle of New Zealand White rabbits using a temperature sensitive liposomal formulation containing lysolipids (Thermodox®, Celsion, Columbia, MD). In this study, a focused ultrasound transducer was scanned mechanically along a defined trajectory while MR-based temperature maps provided a feedback for power control to maintain a target temperature of 43±1°C for 20 minutes. On average a 15 fold increase of doxorubicin in heated compared to non-heated muscle tissue was observed.

Real-time monitoring and quantification of drug delivery

For tumors, the deposited amount of drugs may be furthermore affected by inter-tumoral variations, such as differences in perfusion, vascularization, or the presence of a necrotic core, asking for a tool to quantify drug uptake. One possible route is the incorporation of MRI contrast agents (CA) together with a drug into temperature sensitive liposomes (Figure 2) that is co-released with the drug and allows MR-imaging of the release process [45].

Figure 2: Focused ultrasound is used to non-invasively induce local hyperthermia (ca. 42°C) in the tumor tissue. Temperature sensitive liposomes containing MRI contrast agents together with a drug, e.g. doxorubicin, release their payload when passing through the heated tissue. The release of contrast agents leads to a signal change in MRI and can be used to follow and quantify the drug release (reproduced with permission from de Smet et al.[45]).
As liposomal encapsulated CAs show a low apparent relativity due to the hindered water exchange across the lipid bilayer, the release of the CAs from temperature sensitive liposomal formulations can be imaged with MRI, when heated with focused ultrasound.[56] The application of this concept to image and quantify drug delivery, also termed “chemodosimetry” or “dose painting” was demonstrated in preclinical studies with a temperature-induced release of Mn\(^{2+}\) acting as T\(_1\) agent, which was co-encapsulated with doxorubicin inside heat sensitive liposomes.[57-60] Though most likely not translatable to the clinic due to the toxicity of Mn\(^{2+}\), these studies demonstrated the value of imaging techniques to quantify the drug delivery and uptake in tumors non-invasively. In two recent preclinical studies the clinically approved T\(_1\) contrast agent, GdHPDO3A (Prohance®, Bracco), was encapsulated inside liposomes together with doxorubicin (Figure 2).[44-46] Negussie et al. performed a study with imageable liposomes in VX2 rabbit tumors on a clinical MRgHIFU system that employs a phased array transducer with electronic beam steering capabilities in order to homogeneously heat a target tissue.[44] The release of the MRI contrast agent in the tumor leads to noticeable signal changes that within the heated volume. A similar MR-HIFU system was used by de Smet et al. for a drug delivery study in rats bearing a subcutaneous tumor. In addition, the system was equipped with a designated setup for preclinical studies. In this proof-of-concept study, it was demonstrated that MR-HIFU can be used to induce and maintain stable hyperthermia in a tumor tissue leading to an enhanced drug release. Furthermore, the T\(_1\) contrast change visualized with MRI upon release allowed a non-invasive quantification of the drug uptake in the tumor and revealed well and poorly perfused tumor regions (Figure 3) [45]. These findings may have direct impact on clinical decision making, as right after the intervention the drug uptake in the tumor can be quantified.

Figure 3

Figure 3: Temperature sensitive liposomes containing doxorubicin and an MRI contrast agent were intravenously injected. The tumor was heated for 30 minutes to 42°C using HIFU. T\(_1\) maps
acquired before and after heating show a drop of $T_1$ in the tumor due to the release of the MR contrast agent (left panels), while almost no $T_1$ change is observed in non-heated tumors (right panels). In some tumors a $T_1$ change is only observed in the well vascularized tumor rim, revealing a necrotic core within the tumor (adapted and reproduced from de Smet et al.[45]).

Temperature induced drug delivery using MR-HIFU with subsequent non-invasive drug quantification using MR is an excellent example of image guided intervention. The diagnostic imaging modality MRI takes here a central role in the “See, Reach” as well as the “Treat” phase (Figure 4).

Figure 4

Figure 4: Image guided drug delivery using MR-HIFU. MRI plays a crucial role in the treatment planning, during the treatment for providing temperature and spatial feedback, as well as in an evaluation of the treatment.

Pressure-sensitive drug delivery systems

Ultrasound started to be investigated for new drug delivery applications with the advent of ultrasound contrast agents (UCA), commonly named microbubbles (MBs). These contrast agents, initially developed to compensate for ultrasound insensitivity to detect capillary blood flow, oscillate in the sound field returning distinct echoes used to generate contrast enhanced images. Exceeding a certain ultrasound intensity threshold, the microbubbles loose integrity and burst (cavitation). This ultrasound-microbubble interaction creates physical phenomena such as microjets or shockwaves that permeabilize the surrounding blood vessels and tissues effectively mediating extravasation and cellular uptake of circulating drugs otherwise trapped in the vasculature (Figure 5) [61] [38]. The permeabilization of cell membranes with ultrasound is termed sonoporation. Ultrasound can also be used to induce cavitation effects without
the presence of microbubbles although the pressures at which this is possible are much higher than the ones necessary when microbubbles are used [62].

Figure 5: Microbubbles and drug/gene carriers are injected intravenously. When the microbubbles are exposed to the ultrasound beam they vibrate undergoing volume changes and, if the ultrasound intensity is above a certain threshold, they ultimately cavitate. The forces generated permeabilize the surrounding endothelial cell layer. The co-injected drugs/genes are now able to extravasate into the tissue. Dark circles: drug/gene carriers; Light circles: microbubbles.

Microbubbles

Microbubbles are gas-filled microcapsules stabilized by a biocompatible shell with a mean diameter around 2 μm, able to circulate through the lung capillary bed. The shell can be made of lipids, proteins or biodegradable polymers although currently only protein (e.g., Optison®) and lipid shelled (e.g. SonoVue®, Definity®) agents are approved for clinical use. For lipid and protein based agents the use of hydrophobic gases, such as sulfur hexafluoride or perfluoropropane, is needed to further stabilize the MBs by preventing gas dissolution through its thin shell (few nanometers). Polymer microbubbles have a thicker, harder shell, 20-100 μm, and do not need to use very hydrophobic gases to remain stable in circulation but only generate sufficient ultrasound contrast at pressures higher than the ones needed for lipid- and protein-shelled agents [38]. Nonetheless, microbubbles are very reactive to low ultrasound pressures as they undergo significant volume changes due to the encapsulated gas compression and expansion. As a result of the acoustic impedance difference between the gas and surrounding tissues the acoustic backscatter signal from these microbubbles is higher than the one from blood and soft tissues, of which non-linear components give the most specific information for imaging [63]. In practice, three microbubble oscillation states are recognized to describe its behavior according to the ultrasound pressure amplitude.
applied: stable linear scattering, stable non-linear scattering and transient non-linear scattering and destruction, for very low, low/mean and high pressures, respectively. Once injected, microbubble contrast can be imaged up to 20 minutes for humans and only for a few minutes for mice and rats as microbubbles are rapidly removed from circulation by the reticulo-endothelial system (RES) which includes e.g. liver and spleen [64].

For diagnostic ultrasound imaging typically $10^8$ - $10^9$ microbubbles are injected (normally with repeated bolus injections) and they can be used for instance to improve endocardial border delineation and, thereby wall motion abnormalities, and analysis of myocardial perfusion, which further helps to identify the myocardium at risk. Perfusion imaging using such agents can also be applied to other tissues such as the liver or kidneys [65].

**Pressure-sensitive drug carriers**

Microbubbles can also serve as drug carriers, where local release is triggered with focused ultrasound. Upon cavitation, these drug loaded microbubbles release their payload, while simultaneously inducing sonoporation to mediate drug uptake. As the latter effects are transient in nature and remain only for a limited time, a high local drug concentration is needed. Several concepts have been developed to attach drugs to the bubble shell, to build them into the shell, or to put them in an additional liquid reservoir on the inside of the microbubble [64]. For incorporation in the shell, a polymeric microbubble with a relatively thick shell offers the highest volume for drug incorporation. However, drugs incorporated in the shell do not release quickly or at all when the bubble is destroyed, as they tend to remain associated with the bubble fragments that are formed. [66] For this reason, systems in which drugs are present in an additional liquid reservoir form a more useful delivery vehicle, because half the volume of the microbubble or more can be used, however, this method is only applicable to hydrophobic drugs. Lipid-shelled and polymer-shelled microbubbles with an additional oil-phase to increase the reservoir size to incorporate hydrophobic drugs have also been investigated. [67] [68]. A more flexible method is to have multilayers [69] or attach liposomes to the outside of microbubbles. In this case, both lipid and polymeric
microbubbles [70-72] [73] can be used and both hydrophilic and hydrophobic drugs can be incorporated. The short circulation half life of microbubbles, the fact that after systemic injection only a fraction of them will pass through the target area, e.g. a tumor, and the amount of drug effectively loaded in/on them are still a point of concern for their use as drug carriers [64, 65].

Targeting of microbubbles

The concept of using microbubbles for imaging and drug delivery has been extended to targeted applications. Due to their micrometer size microbubbles remain inside the vasculature and as such endothelial cell surface markers have been researched as possible targets. For this the shell is coated with specific ligands e.g. to target endothelial markers of angiogenesis and/or inflammation [74, 75] using direct coupling of targeting ligands to the microbubble shell with peptide bond formation chemistry [76].

Multiple studies were conducted imaging angiogenesis and anti-angiogenic therapy, via biotinylated antibodies against \( \alpha v \beta_3 \) present in tumor vasculature [77] [78] [79] as well as using targeting streptavidin-carrying lipid microbubbles coupled to biotinylated antibodies against VEGF receptor 2 [80]. Even though the antibody-antigen binding is very strong it is a relatively slow reaction. This is particularly important when the target is situated in an area of fast arterial flow and high shear stress where the antibody-antigen interaction time window is very limited [81]. However, successful coupling of small ligands [82] and antibodies [83] with good yield has been repeatedly demonstrated and recently, a lipid microbubble with an incorporated lipopeptide in the shell was shown to effectively bind to VEGF2 receptors in tumors [84].

Devices for pressure-sensitive drug delivery

Clinical ultrasound imaging systems and transducers generate enough pressure to disrupt microbubbles. A common application is in perfusion-reperfusion imaging of organs, where circulating microbubbles are disrupted with a high intensity pulse
followed by a low intensity imaging mode which allows visualizing blood flow entering the organ as microbubbles circulate into the organ [65]. There are several examples in which clinical ultrasound machines have been used for pressure-induced drug delivery [85] [86]. However, these types of transducers are developed to image full body structures and as such the sound field covers a very wide area (several centimeters) where microbubbles will be destroyed. Although they might be used to sonicate large superficial structures, e.g. subcutaneous tumors, their application for deep structures is hampered due to the fact that microbubbles will be destroyed along the entire path of sonication leading to drug delivery in undesired body tissues.

Specific ultrasound devices for ultrasound triggered and ultrasound image-guided therapy have been developed and are typically composed of a dual transducer set-up [87] combining a focused and an imaging transducer. The focused transducer delivers ultrasound pressure waves in a defined volume, minimizing unwanted effects, such as microbubble destruction, outside the desired treatment zone. Moreover, several parameters such as ultrasound intensity or duration of pulses are easily defined which confer a great deal of flexibility for optimizing and developing delivery protocols. Mostly the frequencies used range between 1-2 MHz (lower frequency = less tissue interaction = more depth), with an approximate focal zone of 1-2 mm in diameter and 6-10 mm in length. The imaging transducer is then used to guide the focused transducer and obtain a wider view around the targeted area and, for instance, to follow the inflow of microbubbles. The combination allows acoustic parameters such as frequency, pressure, and pulse length to be set independently for the therapeutic and imaging ultrasound transducers [88].

Possible risks and side effects are associated with detrimental bioeffects, which high amplitude pressure waves may induce in healthy tissue. The occurrence of tissue hemorrhage and endothelial cell damage after ultrasound exposure of cultured cells and organs containing air, such as the lungs or the intestine have been reported [89][37-39],[90-94]. Simultaneous exposure to microbubbles and high-energy ultrasound resulted in a reversible and transient decrease in left ventricular contractile performance, increase in the coronary perfusion pressure, increase in the myocardial lactate release,
and presence intramural hemorrhage in the plane of ultrasound transmission. Further capillary ruptures, erythrocyte extravasation and endothelial cell damage have been observed [93]. These effects were directly related to the mechanical index, an ultrasound safety parameter defined as the ratio between the peak negative pressure and the square root of the frequency used. These studies indicate that although high-energy ultrasound seems to be necessary to induce tissue permeability facilitating local drug delivery, it may also have significant bioeffects in the myocardium and other tissues. Therefore, careful optimization of the optimal ultrasound parameters to enhance drug delivery with microbubbles is essential [95] [94].

Examples for pressure-induced drug delivery: nucleic acid-based drug delivery

Among the innovative therapeutic field of gene therapy new types of drugs aiming at enhancing or blocking the expression of a target protein are emerging; and they bring additional challenges with them. The selective delivery of plasmid (pDNA) or oligonucleotide DNA (ONDs), and more recently siRNAs (or plasmid based small hairpin RNA interference – shRNA), to cells is increasingly addressed using intravenously administered nanosystems (e.g., liposomes, nanoparticles and nanocapsules). siRNAs or antisense ONDs will not be a substitute for cancer chemotherapy, but will more likely be used in a combination treatment in synergy with chemotherapy or other therapies; the objective will be to control the disease with a much more favorable toxicology profile and e.g. to avoid recurrences after classical treatments where micrometastases may have escaped [96]. RNAi-based drugs have been used to target dominant-mutant or amplified oncogenes, translocation products, signaling molecules and viral oncogenes amongst other gene products. Therapies based upon RNAi may have a number of inherent, fundamental benefits, such as harnessing natural pathways and the potential to target virtually any protein, i.e., no limitation to “drugable” proteins.

However, the in vivo application, especially systemic delivery of nucleic acids, in particular siRNA, is facing challenges from barriers in the extracellular environment and
the intracellular uptake. The first hurdle is the small size of oligonucleotide- and siRNA-based molecules which are rapidly excreted through the kidneys when administrated into the blood stream, even if these molecules remain stable in circulation through chemical modifications. Second, nucleic acids are relatively unstable in the serum environment and they can be degraded by DNase and RNase activity within a short period of time. Third, when they are administered systemically, the nonspecific distribution of these molecules throughout the body will significantly decrease the local concentration where the disease occurs. In addition, they need to overcome the blood vessel endothelial wall and multiple tissue barriers to reach the target cells. Furthermore, when it reaches the target cells, cellular uptake of those nucleic acid molecules activity require efficient endocytosis and in the case of siRNA intact double-stranded oligos. [96, 97]. In the case of shRNA and pDNA they need to penetrate the nuclear membrane as well.

An ideal delivery vehicle for cancer therapy for instance must be able to selectively and differentially target tumors versus normal tissue, homogeneously distribute through the tumor mass and penetrate the tumor cells following systemic administration. However, it still has to (partly) compensate for the above mentioned hurdles of nucleic acids delivery.

Viral vectors are popular for laboratory delivery of pDNA and shRNA because of their high transfection efficiency and effective integration of exogenous DNA, but they have been losing support in recent years because of concerns over safety due to the potential risk of insertion mutagenesis and interference responses [98] and immunogenicity [99, 100].

Non-viral delivery systems, in particular those with biodegradable components, have much better safety profiles than their viral counterparts though their transfection efficiency is generally lower [101] [102].

Many different non-viral vehicles for delivery of siRNA, pDNA and shRNA have been developed in the last few decades to improve stability of such compounds and provide efficient delivery to cytoplasm and nucleus, including cationic liposomes and polymers,
stabilized nucleic acid lipid particles and cyclodextrin-containing polymers, and a number have progressed to the clinics [103-105]. Their positive charge facilitates complexation with negatively charged nucleic acids and also binding to the negatively charged glycocalyx on external cell membranes promoting endocytosis. Polyethylenimines (PEI), as one of the most effective poly-cationic gene vectors, could condense plasmids DNA into cationic polymers, protect the plasmids against being degraded by nucleinase or enzymes within a few hours, and enhance the endocytosis of plasmids DNA, thus promoting gene transfection in vivo [106]. Once endocytosed, the vehicle's positive charge facilitates early escape from the endosome avoiding lysosomal degradation [107]. Though the positive charge of these vehicles improves their transfection efficiency, it is also associated with increased toxicity [108]. Also, transgene expression of intravenously injected complexed pDNA is not limited to ultrasound exposed tissue as it can actively enter cells [109].

Certainly not all of those obstacles can be overcome with ultrasound-mediated delivery but some are favorably influenced (Table 1).

Table 1 Preclinical and clinical delivery of nucleic acids: challenges and solutions

<table>
<thead>
<tr>
<th>Delivery challenge</th>
<th>Delivery solution</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excretion profile</td>
<td>Formulation into nanocarriers with diameters larger than the fenestrations of RES organ vessels</td>
<td>[110]</td>
</tr>
<tr>
<td>Trapping in RES (liver, spleen, lung and bone marrow) leading to degradation by activated monocytes and macrophages</td>
<td>Modification (e.g. PEGylation) to increase circulation time</td>
<td>[111]</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>Serum instability (RNAse degradation and serum)</td>
<td>Chemical modification</td>
<td>[112]</td>
</tr>
<tr>
<td></td>
<td>Encapsulation in delivery</td>
<td>[113]</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>Protein Binding</td>
<td>Nanoparticles</td>
<td></td>
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<tr>
<td>-----------------</td>
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<td></td>
</tr>
</tbody>
</table>
| Non-specific distribution | Enhancement of local uptake through ultrasound and microbubbles  
Targeting of nanocarriers  
Exploitations of angiogenesis and EPR effect  
Enhanced extravasation through US and micobubbles  
Sequence optimization  
Oligonucleotide modifications  
Encapsulation,  
Use of biodegradable, biocompatible, non-immunogenic materials  
Targeting |
| Tissue barriers |  
Immune response (TLR and non-TLR mediated)  
Off-target effects  
With properties of nanoparticle surface such as haemolysis, thrombogenicity, phagocytosis by scavenger macrophages, neutrophils, complement activation  
Enhance cell membrane permeability through sonoporation  
Image guidance for intervention  
Coformulation with imaging |
| Toxicity through properties of nanoparticle surface such as haemolysis, thrombogenicity, phagocytosis by scavenger macrophages, neutrophils, complement activation |  
Off-target effects  
Immune response (TLR and non-TLR mediated)  
Enhance cell membrane permeability through sonoporation  
Image guidance for intervention  
Coformulation with imaging |
| Target cell uptake |  
Enhance cell membrane permeability through sonoporation  
Image guidance for intervention  
Coformulation with imaging |
| Delivery monitoring |  
Enhance cell membrane permeability through sonoporation  
Image guidance for intervention  
Coformulation with imaging |
<table>
<thead>
<tr>
<th>labels (e.g. MRI, optical probes) instead of biopsy and ex vivo analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapy resistance</td>
</tr>
<tr>
<td>Combination of drug treatment with hyperthermia through US-mediated heating</td>
</tr>
</tbody>
</table>

RES: reticuloendothelial system; PEG: polyethylenglycol; TLR: Toll-like receptor

Pressure-induced plasmid DNA delivery has been investigated both in *vitro* and *in vivo* [37, 127] and such examples have been described in cardiology, where local gene delivery has been established in preclinical models both for marker genes as well as for therapeutic genes [36] and further in tumors [31]. Plasmid DNA is a negatively charged, high molecular weight, more than 2500 kDa in size, and synthetic non-viral gene vector too large to escape circulation and enter cells and/or tissues. When injected in its naked form it is subjected to degradation by circulating endonucleases and its serum half-life has been reported to be less than 1 minute in mice [109]. As a result, plasmid DNA transfection *in vivo* is characterized by low efficiency. Complexation of plasmid DNA with cationic lipids or proteins is commonly used as it reduces its negative charge, partly protects it from endonucleases and facilitates cell entry as the cationic complexes interact with cell membranes and endosomes. Although cationic polymers could enhance the gene transfection *in vitro*, the results of *in vivo* studies are not that satisfactory because targeting vectors have to overcome chemical and structural barriers to reach cells [128], however they still have shown to markedly improve gene transfection efficiency in cardiovascular structures and surgical inaccessible tumors [129] [130]. Therefore, non-viral gene transfer has low efficiency *in vivo* and transfection with intravenously administered plasmid DNA is difficult [131]. In addition, both tumor targeting and cell entry, can be enhanced by decoration or complexation of the vehicle with targeting moieties, such as monoclonal antibodies, peptides, small molecule ligands, and aptamers to recognize cell surface markers [132].
Ultrasound-mediated gene delivery using microbubbles to induce localized sonoporation has been investigated as a potential solution to enhance *in vivo* gene transfection efficiency [31, 36, 37, 64].

Table 2 Selected studies of pressure-induced drug and gene delivery

<table>
<thead>
<tr>
<th>Reference</th>
<th>Drug/Gene</th>
<th>Ultrasound Protocol</th>
<th>Microbubbles</th>
<th>Tissue; Animal</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>[133]</td>
<td><strong>Bleomycin</strong> 20 µg/mouse</td>
<td>1 MHz, 2 W/cm², 50% DC for 1 min on days 0, 2, 4, 6, 14, 16, 18, and 20</td>
<td>Optison (1:1 with drug)</td>
<td>Tumor; mice; intratumoral injection</td>
<td>Complete tumor disappearance by 56 days</td>
</tr>
<tr>
<td>[134]</td>
<td><strong>Bleomycin</strong> 0.15 mg/mouse</td>
<td>1 MHz, various DC and intensities for 1 or 2 min; best results are for 3.5 W/cm² CW</td>
<td>None</td>
<td>Tumor; mice; intratumoral injection</td>
<td>Complete tumor regression by day 10. [US w/o drug had no effect; using 35% DC had no effect]</td>
</tr>
<tr>
<td>[135]</td>
<td><strong>Micellar DOX</strong> 2.67 mg drug/kg rat</td>
<td>20 kHz, 0.17 MPa, MI=1.22, CW; 476 kHz, 0.84 MPa, MI=1.22, PRF 20.1 Hz Both once a week for 6 weeks</td>
<td>None</td>
<td>Tumor; bilateral; rats; tail vein injection</td>
<td>50% more DOX delivery at 30 min, but no difference at later time points</td>
</tr>
<tr>
<td>[86]</td>
<td><strong>pDNA encoding TK</strong> complexed with</td>
<td>1.3MHz; MI 1.6; 7MHz for imaging</td>
<td>Lipid microbubbles</td>
<td>Tumor; mice; intravenous</td>
<td>After ganciclovir treatment of TK transfected tumors</td>
</tr>
<tr>
<td>Reference</td>
<td>Treatment Details</td>
<td>Ultrasound Parameters</td>
<td>Microparticle Type</td>
<td>Heart Location</td>
<td>Comments</td>
</tr>
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</tr>
<tr>
<td>[85]</td>
<td>microbubbles; 100μg pDNA; pDNA encoding SCF and hSDF-1α; 0.6mg pDNA/kg</td>
<td>15L8 transducer; MI 1.6; 1xburst every 2000ms; scanned base to apex repeatedly for 20 min; treated 7 days post-infarct and repeated up to 6 times</td>
<td>Definity (0.12mL/rat)</td>
<td>Heart; rat intravenous injection</td>
<td>Up to 6x increase in vascular density in infarct area; higher myocardial perfusion, smaller scar size, higher myofibroblast recruitment (for repeat 6 compared to controls)</td>
</tr>
<tr>
<td>[136]</td>
<td>shRNA - survivin; 50μg/mouse</td>
<td>3MHz, 2 W/cm2, DC 20%, 2 min</td>
<td>Sonovue</td>
<td>Tumor; mice; intravenous injection</td>
<td>Significant increase of caspase-3 and decrease of survivin in treated group compared to controls</td>
</tr>
<tr>
<td>[137]</td>
<td>siRNA – ICAM-1 complexed with microbubbles (20μg/artery)</td>
<td>1MHz, 0.5 W/cm², duty cycle 50%</td>
<td>Optison (10μL)</td>
<td>Femoral arteries; mice; intra-arterial (closed/no-flow) injection</td>
<td>Prevention of neointimal formation after siRNA treatment (5x decrease)</td>
</tr>
</tbody>
</table>

*CW = continuous wave; DC = duty cycle; DOX = doxorubicin; ICAM-1 = intercellular adhesion molecule-1; MI = mechanical index; pDNA = plasmid DNA; PRF = pulse repetition frequency; SCF = stem cell factor; hSDF-1α = human stromal cell derived factor-1α; TK = thymidine kinase; VEGF = vascular endothelial growth factor*
These systems have been successfully employed in ultrasound gene delivery [104] and several groups non covalently attach plasmid DNA to the shell of microbubbles to protect it from degradation (Table 2) [85, 86]. Direct injections of plasmid DNA (e.g. intramuscular or intratumoral) in combination with microbubbles and ultrasound are also used although this more invasive nature is probably not a desired solution particularly for gene transfer in deep tissues. An abundance of literature [138, 139] [37] reported that the combination of cationic polymers and ultrasound could improve transfection efficiency [128]. Surprisingly the reticuloendothelial system seems not to be the limiting factor for the ultrasound-based gene delivery [140]. Our group (unpublished results) has successfully transfected tissues in vivo following intravenous injection of naked plasmid DNA co-injected with polymer microbubbles. Naked plasmid DNA or microbubble bound plasmid DNA has the advantage of having none to very limited transgene expression after intravenous injection without ultrasound treatment.

Finally, shRNA expression vectors, that encode siRNA like sequences, could be delivered by ultrasound-mediated drug delivery systems, but related studies are rather sparse [141] [142] [37]. In a number of studies it was demonstrated that it is possible to deliver siRNA intracellular via microbubble-enhanced focused ultrasound [72, 143] [37, 142].

In summary, ultrasound mediated, image guided drug delivery could synergistically promote the development and application of various existing and to come gene transfer methods in vivo.

Future outlook
The integration of therapeutic interventions with diagnostic imaging, to allow for local image-guided delivery, calls for developments in equipment and agents including new therapeutics. Advances in computer technology, coupled with an increase in the accuracy and sensitivity of imaging technologies, will make it possible to seamlessly integrate diagnosis and treatment. Future image-guided interventions will enable
medical practitioners to detect critical illnesses at their most curable stage – often at the cellular level, before any symptoms are noticeable by the patient. The practice of medicine will shift from one of disease detection and treatment to one of prediction and prevention in asymptomatic, at-risk populations. Focused ultrasound in combination with MRI and US imaging has great potential to bring localized triggered drug release to the clinic, while employing pressure and temperature sensitive delivery vehicles. The preclinical data demonstrate the specific solutions that are emerging for local drug and gene delivery in both oncology and cardiology.

In first approximation, the amount of drug that can be delivered into a target tissue scales with the drug plasma concentration, the tissue perfusion and the length of the procedure. The latter is less of an issue, as hyperthermia can be applied in a clinical setting over a time span of 30 minutes to 1 hour taking also patient comfort into account. In temperature mediated drug delivery, high drug plasma levels can be maintained with temperature sensitive liposomes that show little clearance and as little as possible drug leakage at body temperature. This requirement immediately puts a challenge to further improve temperature sensitive liposomes and drug formulations. One requirement is that drugs are readily taken up in the target tissue upon release as drug uptake competes against down-stream washout with the blood. Drugs like doxorubicin have a pharmacokinetic profile and a high enough free volume of distributions to be a prima candidate for this application. Mainly small molecule drugs will therefore benefit from temperature induced drug delivery as the therapeutic window can be extended with this approach.

For pressure mediated drug delivery, the way forward may be co-injection of microbubbles and drugs. As microbubbles show a short circulation time, binding the drug to the bubble will logically reduce the circulation time of the drug. Co-injection of drugs and bubbles may exploit the fact that ultrasound induced transient bioeffects like induced extravasation and sonoporation have a longer half life compared to bubble circulation. As drug uptake is coupled to these bioeffects rather than the bubble circulation, a higher total drug uptake can be achieved. As pressure induced drug
delivery mediates drug uptake across biological barriers, mainly drugs with hindered
extravasation or cellular uptake will benefit from this effect.

Currently, *in vivo* gene therapy delivery, through either the local or systemic route, is
still mainly serving as a research tool in functional genomics and the proof of principle
for potential gene therapeutics, however lately more and more clinical studies are
pursued. Despite the delivery obstacles that need to be overcome, the significant
advantages of siRNA, plasmid and oligo-based therapeutics are the speed with which
different therapeutic sequences and the matching genes can be studied. Ultimately, the
effort for *in vivo* delivery will be translated into many clinically viable administration
methods to treat various cancer, viral infection, autoimmune and CNS diseases [144].

Such complex, platform-based drug delivery solutions with underlying drug-device
combinations call for intense and precompetitive collaborations and public-private
partnerships as well as for alliances and new business models between the medical
device and drug development industry in an open innovation framework. A number of
cases are already under the way.

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**Executive Summary**

**Image guided interventions and therapy**

Medical imaging technologies are becoming an integral part of therapeutic interventions.

Ultrasound mediated delivery methods are developed to deliver drugs in an inert form to
specific disease sites within the body and then releasing or activating them precisely
where they are needed – for example, in the center of a tumor.

Targeted delivery produces more constant and controlled drug concentration profiles
with favorable pharmacokinetics.

Image-guided techniques are needed to target drugs within the body that are based on
the use of drug-loaded intravenously-injected nanoparticles that inertly carry a drug in the bloodstream, and focused energy beams that rupture these nanoparticles and release the drug when they reach the target tissue.

Imaging enables to monitor the amount of drug released in order to provide real-time therapy delivery and efficacy feedback. Ultrasound and/or MR imaging play an important role in the targeting and monitoring processes and closing e.g. the temperature control loop.

**Temperature and pressure sensitive delivery systems**

Temperature sensitive liposomes with incorporated drugs and MRI-imaging labels as well as pressure-sensitive microbubbles with specific ultrasound-imaging and drug release characteristics are developed for the local delivery of various therapeutics. They are suitable for drugs with different physico-chemical and pharmacokinetic properties

Temperature-dependent systems will mainly support small molecule drugs maintaining high plasma levels and extend the therapeutic window

Pressure-dependent systems with short circulation times will mainly enhance extravasation and sonoporation effects and therefore higher total drug uptake across biological barriers.

Pressure-mediated delivery implies an extension of the current application of ultrasound microbubbles.

**Future perspective**

The specific combination of ultrasound, delivery and contrast vehicles, imaging technology and the medical application will finally determine the future development of image-guided, ultrasound-triggered drug delivery.

The development of image-guided ultrasound techniques that could non-invasively trigger the delivery of existing, well established as well as new drug formats such as RNAi therapeutics at a targeted location opens up exciting possibilities for advancing
personalized medicine.

New options to improve image-guided drug delivery will need new imaging technology, material development and an expansion of the knowledge on mechanisms of uptake of drugs into cells, especially for high molecular weight therapeutics.

For chemotherapy, dose reduction and thereby the increase of the therapeutic window is the differentiating advantage. While for small molecule drugs entry to the cells can be achieved passively, nucleic acids require an uptake mechanism. Here, ultrasound can also be used as the trigger.

A key success factor for the effective translation of new drug delivery concepts into clinical practice are partnerships with leading academic, medical institutions and industrial partners from the drug and device field.

Financial & competing interests disclosure

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First references about temperature sensitive liposome concept


Hyperthermia concept in chemotherapy


Key publication about the phenomena of sonoporation


■ MRgHIFU technology overview

■ Key review of the ultrasound-mediated drug delivery field

■ Very recent review of gene delivery using ultrasound-mediated drug delivery

First in vivo proof of concept of MRIgHIFU drug delivery using thermosensitive liposomes


Recent and extensive reviews of image-guided, ultrasound-mediated drug delivery


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Figure 1: Ultrasound can be employed to non-invasively and locally either raises the temperature inside the body for ablation or to induce hyperthermia. Hyperthermia as such already shows synergistic effects when combined with systemic chemotherapy. Furthermore, hyperthermia can be used to trigger local drug delivery using temperature sensitive drug carriers. Alternatively, the pressure wave associated ultrasound can be used to mediate drug delivery as well, usually in combination with microbubbles.
Figure 2: Focused ultrasound is used to non-invasively induce local hyperthermia (ca. 42°C) in the tumor tissue. Temperature sensitive liposomes containing MRI contrast agents together with a drug, e.g. doxorubicin, release their payload when passing through the heated tissue. The release of contrast agents leads to a signal change in MRI and can be used to follow and quantify the drug release (reproduced with permission from de Smet et al.[45]).
Figure 3: Temperature sensitive liposomes containing doxorubicin and an MRI contrast agent were intravenously injected. The tumor was heated for 30 minutes to 42°C using HIFU. T₁ maps acquired before and after heating show a drop of T₁ in the tumor due to the release of the MR contrast agent (left panels), while almost no T₁ change is observed in non-heated tumors (right panels). In some tumors a T₁ change is only observed in the well vascularized tumor rim, revealing a necrotic core within the tumor (adapted and reproduced from de Smet et al.[45]).
Figure 4: Image guided drug delivery using MR-HIFU. MRI plays a crucial role in the treatment planning, during the treatment for providing temperature and spatial feedback, as well as in an evaluation of the treatment.
Figure 5: Microbubbles and drug/gene carriers are injected intravenously. When the microbubbles are exposed to the ultrasound beam they vibrate undergoing volume changes and, if the ultrasound intensity is above a certain threshold, they ultimately cavitate. The forces generated permeabilize the surrounding endothelial cell layer. The co-injected drugs/genes are now able to extravasate into the tissue. Dark circles: drug/gene carriers; Light circles: microbubbles.