Nanosonotechnology: the next challenge in cancer sonodynamic therapy

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Abstract

Sonodynamic therapy (SDT) is a newly developed anticancer
treatment where ultrasound is used to trigger the cytotoxic
effect of chemical compounds, known as sonosensitizers.
Although SDT is similar to photodynamic therapy (PDT),
SDT activates the chemical compounds through energy
transfer using ultrasound rather than light. Moreover, SDT
can focus the ultrasound energy onto malignant sites situa-
ted deeply within tissues, thus overcoming the main draw-
back linked to the use of PDT. Several physical and chemical
mechanisms underlying ultrasound bioeffects and anticancer
SDT take advantage of the non-thermal effect of acoustic
cavitation generated by selected pulsed or continuous ultra-
sound. As the physical-chemical structure of the sonosentizer
is essential for the success of SDT, we believe that the dif-
ferent aspects related to nanotechnology in medicine might
well be able to improve the triggering effect ultrasound has on
sonosensitizing agents. Therefore, the aim of this review is to
focus on how nanotechnology might improve this innovative
anticancer therapeutic approach.

Keywords: cancer; nanoparticles; sonodynamic therapy;
sonosensitizers; ultrasound.

1. Introduction

Ultrasonic waves are commonly described as waves that
transport mechanical energy through the local vibration of
particles without any net transport of the particles them-
selves. The term "ultrasound" is generally taken to mean
that the frequency of the wave is greater than the upper
threshold limit human hearing can detect. Therefore, ultra-
sound is a mechanical wave with periodic vibrations of
particles in a continuous, elastic medium at frequencies
≥ 20 kHz [1].

Nowadays, ultrasound is commonly used in most biomedi-
cal applications, both in the field of diagnosis and therapy,
requiring better localization/focusing and higher frequencies
(Table 1). However, even if ultrasound is generally considered

"safe," as no harmful effects have been associated with their
medical use, it can affect tissue through a variety of mecha-
nisms, mainly thermal or non-thermal, depending on various
factors such as the intensity of the beam and the duration of
exposure and frequency (American Institute of Ultrasound in
Medicine) [1].

There are several underlying mechanisms in ultrasound,
i.e., streaming and radiation forces, but the most prominent
mechanical effect on tissue is acoustic cavitation, which is
defined as the formation or activity of gas- or vapor-filled
cavities (bubbles) in a medium exposed to an ultrasound
field [2].

Nanotechnology is defined as the study and use of struc-
tures with a size that varies between 1 and 100 nm. The last
few decades have witnessed an increase in the ability to
manage nanosized materials, consequently, a world of pos-
sibilities in a variety of industries and scientific endeavors
has been opened. As nanotechnology is essentially a set of
techniques that allow for the manipulation of properties on a
very small scale, it may have many and far-reaching applica-
tions. Indeed, one such important area is that of "nanomedi-
cine," which, according to the National Institute of Health
Nanomedicine Roadmap Initiative, refers to highly specific
medical interventions at a molecular level for the diagno-
sis, prevention, and treatment of diseases [3]. Drug-delivery
nanosystems make for a significant portion of nanomedicine.
The importance of nanotechnology in drug delivery is in the
concept and ability to manipulate molecules and supramo-
lecular structures to produce devices with programmed func-
tions. Clinically useful drug-delivery systems must be able to
deliver a therapeutically effective amount of a drug, which is,
more often than not, to be done over an extended period. The
interaction of drug carrier systems with the biological envi-
ronment is an important element on which to base and design
strategies. These systems should be independent in the envi-
ronment and selective at the pharmacological site. If appro-
priately designed, nanoparticles not only may act as a drug
vehicle capable of targeting tumor tissues and/or cells but
also offer a protective barrier against drug inactivation during
transport. Therefore, the purpose of this review is to focus on
how nanotechnology and, in particular, nanoparticles might
improve the sonodynamic therapy (SDT) of cancer.

2. Acoustic cavitation

The non-thermal interaction of ultrasound with bulk liquid
is accompanied by the quite unique phenomenon of cavita-
tion, which leads to an enormous concentration and conver-
sion of the diffused sound energy. Cavitation has at least

Table 1   Frequency ranges and associated applications.

16 Hz–20 kHz	Human hearing
20–100 kHz	Conventional power ultrasound
20 kHz–3 MHz	Sonochemistry
1–3 MHz	Physiotherapy
3–30 MHz	Diagnostic ultrasound

three discrete stages: nucleation, bubble growth and, under proper conditions, implosive collapse. The dynamics of cavity growth and collapse are strikingly dependent on the local environment; cavity collapse in a homogeneous liquid differs greatly from cavitation near an extended liquid solid interface [4, 5]. Cavitation has been classified into two types: non-inertial and inertial. Non-inertial cavitation bubbles oscillate about an equilibrium radius and often persist for many acoustic cycles. These oscillations create a streaming of surrounding liquid and mechanical stresses mix the medium. Inertial cavities are gas bubbles that grow to near-resonance size and may expand to a maximum, before collapsing violently (Figure 1). The temperature and pressure on the material contained within the imploding cavities can reach values >10,000 K and 800 atm. These extreme conditions may induce a multitude of chemical reactions (sonochemistry) both within and surrounding the bubble, including a concentration of energy that can generate light, an emission known as sonoluminescence [6].

Therefore, the wide range of application of acoustic cavitation also includes the direct interaction between the ultrasound and the biological membrane, modifying their permeability [7]. Indeed, ultrasound seems to be capable of modifying drug distribution in the human body, and it is hoped that a better understanding of such mechanisms will result in the optimization of drug therapies. Recently, numerous reports have been published on the application of ultrasound energy to target and/or control drug release in the fields of thrombolytic agents, transdermal drug delivery, anticancer drugs, and gene therapy [8]. Therefore, applying acoustic cavitation in an effort to increase the drug bioavailability is a very promising approach to drug-delivery systems and can be applied in different ways. First, ultrasound energy has the capacity to help agents penetrate through various tissues. Acoustic force is able to drive materials deeply into the skin, blood clots, or
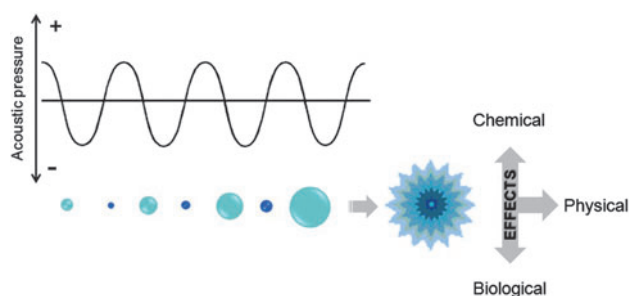
Figure 1   Drawing showing the formation, growth, and collapse of a cavitation bubble.

other tissue. Researchers have hypothesized that these microjets, or the so-called microstreaming, could be applied to promote the diffusion of drugs into various tissues and lesions [7–9]. Indeed, acoustic energy exposure to specific cells by ultrasound, or shock waves, produced by lithotripters, have demonstrated an enhanced uptake of drugs within cells [8]. Another aspect is the use of the microbubbles to carry various drugs to target sites then rupturing the microbubbles by localized ultrasound energy. Indeed, albumin microbubbles were first used to further enhance the effects of thrombolytic agents when used in conjunction with ultrasound [10]. At moderately high sound pressure, amplitudes at the acoustic pressure waves can cause the rupturing of coated microbubble shells, allowing the bubbles to behave as non-coated microbubbles until they diffuse into the bloodstream. Moreover, drug-filled or drug-coated microspheres containing a therapeutic compound may be targeted to specific tissues through sonic energy that is directed to the target area and ruptures the microspheres, releasing the therapeutic compound. Even if the exact mechanism is still unknown, it is likely that the most exciting application of this method could well be gene therapy [11]. It is believed that once microspheres are ruptured, they can create a local increase in membrane fluidity, thereby enhancing the cellular uptake of the therapeutic compound [8]. Furthermore, targeted drug delivery assumes particular importance where drug toxicity is to be taken into careful consideration.

Ultrasound not only can enhance the drug bioavailability *in vitro* and *in vivo*, but they can also change the chemical property of the drug itself. It has been reported that light-sensitive materials, such as non-toxic agents such as hematoporphyrins, can be activated by ultrasound and may kill cancer cells [12]. Researchers discovered that non-thermal ultrasound may activate a group of chemicals that were originally used as light-activated chemicals for cancer therapy through acoustic cavitation, and this innovative ultrasound therapy has been termed "sonodynamic therapy" [8].

3. Sonodynamic therapy

SDT is one of the non-invasive therapeutic applications of ultrasound that has been considered in the last few decades [8, 13–15]. Although SDT is currently under investigation by researchers across the globe, this therapeutic approach is still in its early stage. Cytotoxicity is induced in SDT after the accumulation of a sonosensitizing agent in tumor cells by ultrasonic exposure through the activation of the sonosensitizer. As dedicated acoustic lenses can focus ultrasound into a limited focal zone, they are able to penetrate into the non-superficial tissues. The extent of the focal zone and penetration depth of ultrasound depend on both the focusing grade of the lens and the wave frequency [16].

Heat and mechanical effects are the most common bioeffects associated with ultrasound exposure and are both intensity and frequency dependent [14]. Therefore, the acoustic parameters for ultrasound therapy must be selected with care, taking the objective into consideration. The ultrasonic "level" may be determined by intensity, and Yu et al. [14] suggested

2 W/cm^2 as the critical value between low- and high-level ultrasound. Thus, ultrasound waves, which mostly induce structural alterations, have a high-level intensity, whereas those that mainly modulate tissue functions have a low level [14]. The thermal applications of ultrasound, such as high-intensity focused ultrasound (HIFU) [17], have been more extensively studied than has the therapeutic use of the non-thermal effects of ultrasound, even though it has been reported that free radical scavengers and antioxidants, such as mannitol, vitamins C and E, and superoxide dismutase, could reduce cavitation-induced tissue damage [18].

One way to minimize heating is by using pulsed (e.g., shock waves), rather than continuous ultrasound [19, 20]. Generally, a high-energy shock wave (HESW) can be described as a single pulse with a wide frequency range (up to 20 MHz), high-pressure amplitude (up to 120 MPa), low tensile wave (up to 10 MPa), small pulse width at -6 dB, and a short rise time (<10 ns) (Figure 2). The energy density (up to 1.5 mJ/mm^2) and the pulse energy (up to 100 mJ) determine the temporal and spatial distribution of the pressure profile. The energy density describes the maximum amount of acoustical energy, which is transmitted through an area of 1 mm^2 per pulse [15]. Mechanical stresses seem to play a major role in high-energy shock waves, where the acoustic pressure, frequency of the applied ultrasound, pulse duration, and most importantly, the extent of nucleation of the exposed medium influence the occurrence of inertial cavitation. The likelihood of nucleation increases as the ultrasonic pressure amplitude increases.

As the sonosensitizers used in SDT have no or very low cytotoxicity without ultrasound stimulation, the cytotoxic effects of SDT only occur in the area where the localized ultrasound is applied (Figure 3). SDT, whether pulsed or continuous ultrasound, is considered to be safer and more readily accepted by the patient than either radiotherapy or chemotherapy, which often give rise to source concerns of safety and side effects [8]. SDT differs from photodynamic therapy (PDT), as drug activation is achieved through ultrasound rather than light. Moreover, SDT is able to overcome the main drawback of PDT, i.e., the relatively limited penetration of
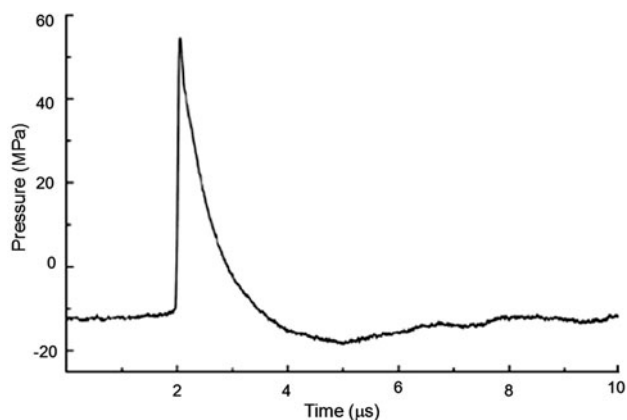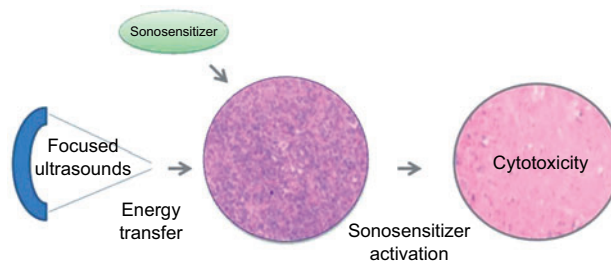

Figure 3 Anticancer SDT: cytotoxicity in tumor tissue when a sonosensitizer as been activated by focused ultrasound (hematoxylin and eosin staining of DHD/K12/TRb tumors photographed under light microscope at a 10× magnification [20]).

light into human tissue, and even at the longer wavelengths, light can penetrate no more than 5 mm into tissue. This goes without saying that this limits such treatments to surface (topical) applications, limiting PDT's application on superficial tumors, those that are directly underneath the skin surface, or on the surface of internal organs. Furthermore, in SDT, ultrasound may be used as an external remote control over time, dose, and location of drug release, providing tailor-made and localized cancer therapy [13].

A series of *in vitro* and *in vivo* trials confirmed that SDT, where either ultrasound or sonosensitizer had no or very low cytotoxicity, was efficacious in destroying malignant cells/tissues, and most sonosensitizers are either porphyrins or their derivatives (Table 2) [16, 21–27].

Protoporphyrin IX (PPIX) is an efficient hydrophobic tumor-localizing agent [28] that can be activated by both light and ultrasound [29]. The subsequent interaction of activated PPIX with molecular oxygen produces a cytotoxic reactive oxygen species (ROS), particularly singlet oxygen, which leads to an irreversible destruction of the target tissue. As the activation of the sonosensitizer is dependent on the cavitation process; high intensity ultrasound is a must. This followed the assumption that ultrasound energy might cause electronic excitation of porphyrins by energy transfer and initiate a photochemical process resulting in the formation of the cytotoxic singlet oxygen (Figure 4). Moreover, as opposed to anticancer drugs, porphyrins are non-toxic in the absence of ultrasound.

Unfortunately, some sonosensitizers have physical-chemical proprieties that seriously limit their clinical applications, such as poor water solubility. This is mainly true of natural products, such as hypocrellin, and nanotechnology is one method that can be adopted to solve such problems [27].

Figure 2 Pressure waveform of a shock wave as a function of time.

Table 2 Porphyrin-related sonosensitizers.

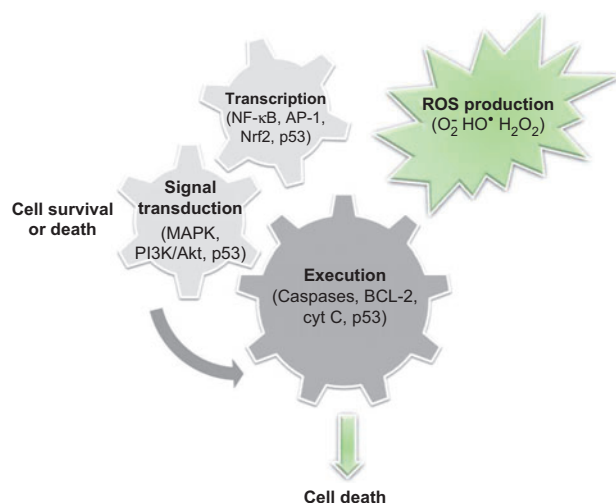| Sonosensitizers	Ref.	Sonosensitizers	Ref.
ATX-70	[21]	Photoprin II	[22]
ATX-S10	[22]	Phthalocyanine	[25]
Chlorin-e6	[23]	Protoporphirin IX	[24]
DCPH-P-Na	[22]	SF1 and SF2	[26]
Hematoporphyrin	[24]	SL052	[27]

Figure 4 Redox-sensitive proteins that can regulate the cell fate decision through the regulation of signal transduction, transcriptional regulation, or execution. Not only does oxidative stress serve as a type of stimulus to trigger stress-response signal transduction pathways, but it can also modulate cell death/survival through direct oxidative modification of those signal molecules. ROS include radical species, such as superoxide (O_2^-) and hydroxyl radical ($HO^\bullet$) along with nonradical species, such as hydrogen peroxide (H_2O_2).

Generally, nanoformulation plays an important role in improving drug delivery by making the drug more water-soluble without changing its physical-chemical structure. Furthermore, nanomodification allows the drug to be delivered to some deeply seated malignant sites that are not easily accessible to drugs. Therefore, we believe that nanotechnology approaches will improve SDT considerably.

4. Nanoparticles as a sonosensitizer delivery system

The tissue distribution of sonosensitizers is agent dependent and thus gives rise to several drawbacks in the bioavailability of sonosensitizing agents, as the therapeutic concentration of the chemical in a specific tissue may not be reached. That is to say, the concentration gradient between the malignant tissue and its adjacent normal tissue may not be sufficiently high to safely carry out SDT and the concentration in the target tissue might be lower than that in plasma, resulting in hemolysis and blood cell rupture. Therefore, the clinical application of today's sensitizers is not free from problems. Indeed, as most sonosensitizer molecules are hydrophobic and are consequently easily aggregated in aqueous media, the sonosensitizer aggregation decreases its efficacy for singlet oxygen production. Moreover, the aggregated sonosensitizer cannot be simply injected intravenously.

Yang et al. [30] describe an oral form of nanoparticles that encapsulate 5-aminolevulinic acid (5-ALA), a precursor of the sonosensitizer PPIX. Chitosan was mixed with sodium tripolyphosphate, and 5-ALA loaded chitosan nanoparticles (CNA) through the addition of 5-ALA with different pH values and concentrations. The results revealed that particle size with different ζ-potential could be manipulated by the addition of 5-ALA with different concentrations and pH values. CNA particles, prepared at pH 7.4 and pH 9 of 5-ALA solutions at a concentration >0.5 mg/ml, showed a promising loading efficiency of up to 75% and an optimum average particle size of 100 nm.

The triblock copolymer, Pluronic P105 (BASF, Mount Olive, NJ, USA), has been found to be an ideal ultrasonically activated drug-delivery vehicle [31]. The copolymer forms dense micelles with strongly hydrophobic cores at concentrations of 4 wt% or above and the polypropylene oxide core is sufficiently hydrophobic to sequester hydrophobic compounds. These micelles have been shown to release their contents upon the application of low-frequency ultrasound, suggesting that drug molecules can be delivered specifically to the insonated regions. Furthermore, once the ultrasound is turned off, the drug is quickly re-encapsulated inside the core of the micelles, preventing the spread of drug outside the targeted region [31]. The circulation time of the encapsulated drug is increased by polyethylene glycol (PEG) chains and prevents the carrier from being cleared by cells in the reticuloendothelial system. Indeed, pluronic micelles are large enough to escape renal excretion but small enough to extravasate at the tumor site. As pluronic has been shown to be non-toxic at low concentrations, it has been used to overcome multidrug resistance. Husseini et al. [32, 33] used an ultrasonic exposure chamber with real-time fluorescence detection to measure the release of doxorubicin from unstabilized and stabilized Pluronic P105 micelles through ultrasonic stimulation. Ultrasonic intensity increases the amount of drug release and frequency decreases it. Such ultrasonically controlled release has been effective against cancer cells both *in vitro* and *in vivo* [31, 34]. It is noteworthy that the amount of release is not a linear function of ultrasonic intensity. In particular, there is a threshold at 0.3 W/cm². Observations of thresholds associated with cavitation and other ultrasonic phenomena are not unusual, and the existence of a threshold for the onset of inertial or collapse cavitation has been extensively documented [35, 36]. Other authors [1, 37] have calculated the threshold for the onset of inertial cavitation as a function of frequency and bubble size. Ultrasonic intensity thresholds reported for an observed biological effect [1, 11, 35, 38] usually differ from thresholds for collapse cavitation and vary according to the type of cell or tissue and the mechanism producing the effect (cell permeability, DNA delivery, cell lysis, or tissue damage) [31]. Husseini et al. [31] suggest that the increase in background acoustic emission, due to the wide spectrum of frequencies of shock waves (white noise), establishes the connection among collapse cavitation, the appearance of the subharmonic, and drug release from the micelles. Therefore, collapse cavitation may be involved in drug release, most probably through high shear forces that disrupt the structure of the micelle.

Drug delivery through nanoparticles is not the only approach that can enhance SDT (Figure 5). Indeed, the existence of a particle in a liquid provides a nucleation site for the cavitation bubble and, due to its surface roughness, leads
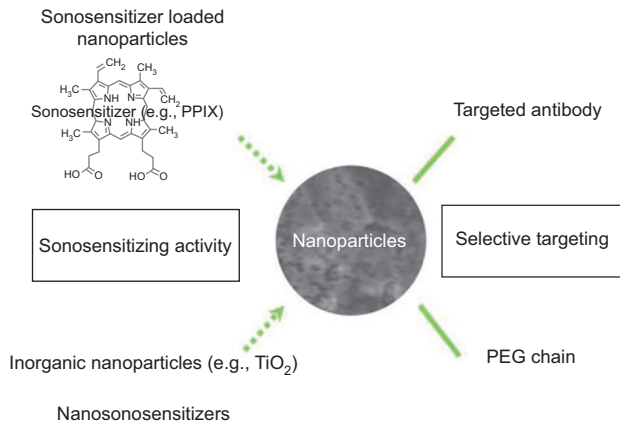
Figure 5 Nanoparticles for sonosensitizing activity as sonosensitizer drug-delivery system and as nanosonosensitizers and for selective targeting as antibody-targeted and PEG-grafted nanoparticles.

to a decrease in the cavitation threshold responsible for the increase in the quantity of bubbles when the liquid is irradiated by ultrasound. Therefore, in this context, another nanotechnology approach might be based on providing the nucleation sites that participate in the formation of cavities to reduce the threshold intensity required for cavitation [39].

5. Nanosonosensitizers

The addition of particles to a liquid, in the appropriate size and amount, leads to an increase in cavitation bubbles and liquid temperature once ultrasound is applied. This suggests that the enhancement in the yield of sonochemical reaction by appropriate particle addition comes from an increase in the number of cavitation bubbles. Thus, the article of Tuziuti et al. [39] clarifies the fact that particle addition has the potential to enhance the yield of the sonochemical reactions, leading to an increasing interest in using nanoparticles as sonosensitizers. Moreover, nanomaterials have some features that make them particularly suitable for SDT from a pharmacological point of view, i.e., their being hydrophobic, having an enormous surface area that can be modified with functional groups that have a diverse array of chemical or biochemical properties, and a subcellular and submicrometer size that allows the nanoparticles to penetrate deeply into tissues with ease and be efficiently taken up by the cells (Figure 5). For example, gold nanoparticles are useful for SDT owing to their special optical properties. The low toxicity, good uptake by mammalian cells, and antiangiogenetic properties of gold nanoparticles make them highly attractive for medical applications [40]. Again, as inorganic nanoparticles often interact strongly with light, they can be used as therapeutic agents and diagnostic techniques for SDT. Indeed, research in this field has been carried out on the use of nanoparticle titanium dioxide (TiO_2) [41]. It is well known that, upon exposure to ultraviolet (UV) light, TiO_2 particles can produce electrons, which lead to the formation of ROS, such as hydrogen peroxide, hydroxyl radicals, and superoxides. Furthermore, TiO_2 was directly activated

under the irradiation of UV and the potent toxicity of TiO_2 has been combined with UV irradiation for use in melanoma C32 cells *in vitro* [41]. Moreover, it has been shown to inhibit tumor growth in a mouse xenograft model. However, the use of nanoparticle TiO_2 still has some drawbacks, such as insufficient selectivity and low efficiency due to lack of cell-specific accumulation of the particles in cancer cells [41]. Xu et al. [42] reported that the combination of the electroporation and the conjugation of TiO_2 with the monoclonal antibody could improve the photo-killing selectivity and efficiency of photo-excited TiO_2 on cancer cells in PDT. Chihara et al. [43] found that liposome-encapsulated TiO_2 might be more effective than non-coated TiO_2 in the treatment of bladder cancer, and these modified TiO_2 might also be a more effective material for SDT because ultrasound irradiation is an alternative energy source for TiO_2 [43]. The recent clinical introduction of HIFU, the non-invasive thermal ablation method that can be targeted to tissues without damaging surrounding normal structures, could be applied as an alternative method to locally irradiating tumors in combination with TiO_2 [41]. Yamaguchi et al. [44] hypothesized that sonocatalytic TiO_2 might become a new tool in SDT for various cancers involving malignant glioma and developed a novel water-dispersed TiO_2 nanoparticle, modified by PEG, demonstrating its photocatalytic antitumor effect on a glioma cell line. These nanoparticle compounds are approximately 50 nm in diameter and do not cross the blood-brain barrier (BBB) in the normal brain. On the contrary, the accumulation of the particles in malignant glioma by transvascular delivery can expected because the normal BBB is disturbed in the tumor site [45]. As the long-term side effects of inorganic nanoparticle accumulation in body organs are still unknown, luminescent silica nanoparticles that decompose in an aqueous solution over a period of hours have been developed [46]. Furthermore, Osminkina et al. [47] reported an *in vitro* anticancer effect of silicon nanocrystals used as a sonosensitizer.

6. Complementary approach: nanoparticles for photoacoustic imaging

Molecular imaging is a new biomedical research field that enables the visualization, characterization, and quantification of biological processes that take place at the cellular and subcellular levels within living tissues, including imaging modalities, such as that of photoacoustic and ultrasound imaging [48]. Molecular imaging always involves an accumulation of contrast agent in the target site; therefore, it has become evident that several of the challenges facing molecular imaging may be overcome by use of nanostructured materials such as nanoparticles. However, for physical reasons, nanoparticles have no role in ultrasound imaging because the frequencies necessary to detect particles smaller than 1000 nm diameter exceed 30 MHz, and when these are >30 MHz, they do not penetrate into living tissues for more than a few millimeters. Furthermore, contrast media are based on air-liquid interfaces of high curvature (bubbles), and the creation of canonized bubbles is no easy feat. In ultrasound imaging, this

gave evidence to the principle that molecular imaging can be achieved using microparticles (microbubbles, with diameters generally larger than about 5 μm) [49]. Ultrasound generated by optical stimulation of structures within a living tissue and nanoparticles may have important roles in "photoacoustic imaging" (PAI). Near-infrared optical imaging and PAI are both strong candidates when dealing with structures that are close to the surface, such as peripheral arteries and veins, which are of considerable interest in the screening of atherosclerosis [50]. PAI, sometimes called optoacoustic or thermoacoustic imaging, is a promising novel imaging modality for the non-invasive visualization of semitransparent objects, including soft biological tissues and biological samples. When short pulses of electromagnetic radiation, such as light or radio waves, illuminate a semitransparent object, a sound wave is induced at the point of interest deep within the sample *via* the thermoelastic effect [50]. The goal of PAI is the recovery of the spatially varying distribution of the absorbed energy inside the illuminated sample from the acoustic pressure measured outside the sample. As structures of different biological tissues reveal highly varying optical absorption coefficients, PAI makes a good distinction between malignant and healthy tissue.

Moreover, in the foreseeable future, it is expected to be useful in the early detection of cancer and blood-rich lesions *in vivo* and it is also expected to provide high resolution in microscopy [50]. Cancer detection using PAI with endogenous chromophores (e.g., hemoglobin and melanin) is an area of active research. One such report is on PAI that used to monitor melanoma tumor growth over the course of 2 weeks [51]. Optical contrast was provided by a higher concentration of melanin in the tumor compared with the surrounding tissue. PAI can also provide information on angiogenesis or vascular changes [52–54].

PAI systems have not only been used in the imaging of melanin and blood vessels but also in the measurement of blood oxygen content by comparing the photoacoustic signal strength of HbO_2 and Hb obtained from spectroscopic photoacoustic images to study hypoxia in tumors, a negative prognostic factor often linked to therapy resistance [55, 56].

PAI has also been used to detect circulating tumor cells in the bloodstream, with the goal of detecting metastasis with contrast agents targeted to circulating tumor cells [57, 58]. Indeed, PAI has been shown to have the potential to identify, visualize, and track tumors and their associated vasculature with high resolution [59]. The sensitivity of the PAI technique to image deeply situated tumors can be further enhanced by exogenous contrast agents. Near-infrared-absorbing dyes, such as IRDye800CW [60], AlexaFluor 750 [61], and indocyanine green [62], have been used to enhance photoacoustic contrast.

However, among the various exogenous contrast agents, gold nanoparticles have attracted special attention due to their unique optical properties from the surface plasmon resonance effect, i.e., gold nanoparticles have an absorbance that is orders of magnitude higher than that of near-infrared dyes. For example, gold nanospheres, nanorods, nanoshells, nanocages, and nanobeacons have been used in PAI because of

their tunable and strong longitudinal plasmon resonance in the near-infrared spectrum [59]. Specific molecular information regarding tumors can be obtained by attaching targeting moieties to exogenous agents. Gold nanoparticles with different optical absorption properties can be conjugated to cancer-specific biomarkers, such as growth factor receptors and integrins. Multiple targeted gold nanoparticles allow for multiplex molecular labeling of a tumor and multiwavelength PAI can evidence the heterogeneous accumulation and interaction of gold nanoparticles with cancer cells *in vivo* [59].

Recently, emphasis has been placed on multimodal nanosystems that can enhance contrast in two or more imaging modalities, including nanowontons consisting of a ferromagnetic (cobalt core) core coated with gold for biocompatibility and a unique shape that enables optical absorption over a broad range of frequencies. The magnetic core acts as a magnetic resonance imaging contrast agent and the gold coating provides the optical absorption contrast for the PAI [63]. The evolution of combined imaging strategies foresees a prominent role in cancer detection and treatment for multimodal nanostructures. Indeed, imaging techniques also have significant roles in cancer therapy, from precise planning and guiding to the evaluation of efficacy. PAI has shown a particular potential in therapies by providing a sequential monitoring of tumor functional properties, such as changes in the tumor vasculature before, during and after therapeutic procedures. Due to their high optical absorption properties in the near-infrared region, the therapeutic agents used for PDT can also act as photoacoustic contrast agents, e.g., PPIX for PDT [29] and gold nanoparticles for photothermal therapy [64].

PAI has an enormous potential in guiding therapeutic procedures and could well provide oncologists with structural and functional information on tumors to facilitate personalized therapy by customizing the therapy dose. Moreover, PAI can also be of help in customizing drug delivery with the use of multifunctional nanoparticles. An example of such a nanoagent is a silver nanosystem involving a poly(lactic-co-glycolic acid) (PLGA) polymer core and outer silver cage network. The outer silver cage enhances contrast in PAI, and the inner core of the nanosystem contains the drug doxorubicin. Preliminary studies have indicated that the PLGA-based nanosystems have the potential to increase contrast significantly in PAI while simultaneously delivering customized payloads of drug to the tumor cells [65]. Another example of controlled and customized drug delivery is that of the use of light-triggered nanoconstructs, such as microspheres containing the drug paclitaxel encapsulated in hollow gold nanospheres. Depending on the concentration of gold nanospheres in the tumor, the light dose can be adjusted to allow for the correct release of paclitaxel [66].

7. Summary and future directions

Even though early results are promising [10, 27, 31, 44, 67, 68], perspectives are encouraging, and nanotechnology and SDT hold out the possibility of a cancer therapy, to date, nanosonotechnology is still in its early stage. Indeed, further

research, both *in vitro* and *in vivo*, is required before it can be considered a clinical therapeutic tool against cancer. However, it has been demonstrated that nanoparticles can improve sonosensitizer bioavailability [30] and cancer targeting [69], opening new horizons in the development of a next generation of nanosonosensitizers, such as gold nanoparticles that carry PPIX [68]. As PPIX acts as a classical sonosensitizer and the gold nanoparticles increase the cavitation rate [70], it might be feasible to suppose that a synergistic effect on tumor cell killing is enhanced by the capability gold nanoparticles to increasing the amount of PPIX in the tumor cells. Indeed, in the presence of gold nanoparticles, the relaxation time of PPIX is longer than the time required for PPIX without gold nanoparticles [71] and favors an efficient generation of singlet oxygen.

Gold nanoparticle-PPIX conjugate has a greater potential than does PPIX alone as a sonosensitizer in the treatment of CT26 tumors by SDT. Such a finding can be related to a higher uptake of PPIX by the tumor cells through its nanoparticle carriers and the presence of gold nanoparticles as an enhancer of cavitation bubbles [67].

Furthermore, the new generation of PAI nanosonosensitizers might improve the therapeutic tools used against cancer. However, despite recent progress, optically active inorganic nanoparticles have not yet achieved broad clinical implementation, possibly due to the fact that drug loading is typically limited to the nanoparticle surface and there is still some concern about long-term safety, prompting further necessary investigation into the toxicity of inorganic nanoparticles [64, 72]. Conversely, organic nanoparticles have found many human therapeutic applications due to their strong biocompatibility and drug-delivery capacity [73]. However, as these organic nanoparticles generally do not intrinsically absorb light in the near-infrared region, they have been of limited use for biophotonics. This led Lovell et al. [74] to introduce porphysomes, organic nanoparticles that are self-assembled from phospholipid-porphyrin conjugates and exhibit liposome-like structures and loading capacity, high absorption of near-infrared light, structure-dependent fluorescence quenching, are enzymatically biodegradable and have an excellent biocompatibility. Porphysome are formed by supramolecular self-assembly, and the subunits consists of porphyrin-lipid conjugates generated by an acylation reaction between lyso-phosphatidylcholine and pyropheophorbide, a chlorophyll-derived porphyrin analogue. This hydrophobic chromophore was positioned in place of an alkyl side chain, maintaining an amphipathic structure. A concentration of 5 mol% PEG-lipid was included in the formulation to enhance *in vivo* pharmacokinetics. Moreover, it has a stabilizing effect because PEG maintained their size and monodispersity for at least 9 months [75]. Porphysome are spherical vesicles measuring 100 nm in diameter, with two layers of higher-density material separated by a 2-nm gap, corresponding to two separate monolayers of porphyrin. They can form metal-chelating bilayers and are useful in a scenario where specific operating wavelengths are required. As porphysomes are higly self-quenched, energy that would normally be released to fluorescence and singlet-oxygen generation is dissipated

elsewhere [74]. Porphysomes are intrinsically suited for multimodal imaging, such as photoacoustic tomography, and, together with other gold and magnetic nanoparticles, is promising approach in non-invasive detection of circulating cancer cells in blood vessels [76] as well as in sentinel lymph nodes [77]. In conclusion, it may be inferred that nanotechnology and SDT are two valuable approaches that can slow down the process of tumor growth, but the combination of "nanosonotechnology" might open an innovative and more effective scenario in cancer therapy.

Acknowledgments

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