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Using ultrasound to formulate nanotherapeutics

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ABSTRACT

The structure of drug particles, and not only their molecular composition, plays an important role in determining their biological fate and therapeutic efficacy (1). Ultrasound is a promising technology that enables controlling particle size at the micro- and nanoscale (2). Furthermore, ultrasound can be used for separating and concentrating particulate sub-populations according to their dimensions and physical properties. The ability to tune the frequency and amplitude of ultrasonic waves makes this technology attractive for dealing with poorly soluble drug candidates and ensures structural integrity when formulating delicate biomolecules, such as proteins and nucleic acids.

INTRODUCTION

Advancements in the fields of pharmaceutical chemistry and synthetic biology are generating new classes of therapeutic compounds. In many cases, formulating these compounds into aqueous dispersible particles is a prerequisite for proper in vitro and in vivo evaluation. Drug formulations can protect compounds from degradation, increase solubility, and extend circulation time. Advanced formulation technologies are also capable of targeting specific tissues and regulating the drug release profile at the target site. Formulating a drug usually requires co-surfactants for stabilizing the final package. In addition, a source of energy, such as ultrasound, milling, extrusion or microfluidics, is necessary many times to control particle size. This prospective discusses the important role ultrasound is playing in fabricating micro- and nanoscale drug formulations.

PARTICLE SIZE AFFECTS BIODISTRIBUTION

The size of drug particles/formulations affects their biodistribution. In humans, nanoscale drug delivery systems, injected intravenously, target tumors and reduce side-effects by circumventing functional healthy tissue such as the heart or kidneys (3, 4). Microparticles (>1000 nm in diameter), have a prolonged residence time in the lungs and synovial joints (5, 6); however, exceeding 15 μm in diam. will clog the pulmonary capillaries when injected intravenously (7). Extremely small nanoparticles (<15 nm in diameter) can ferry drugs across the blood brain barrier, while larger nanoparticles exhibit exponentially decreasing brain permeability. Nanoparticles administered subcutaneously are trafficked to the lymph nodes within an hour (8), while microparticles injected intramuscularly or intraperitoneally will reside onsite, releasing a drug locally, for prolonged periods of time (9).

PARTICLE SIZING TECHNOLOGIES

Several technologies have been developed to control particle size. These can be divided into two categories: technologies that require direct contact with the manipulated matter (such as: milling, extrusion, and microfluidics), versus technologies that do not require direct contact with the matter (such as ultrasound). Avoiding direct contact can reduce batch-to-batch cross contamination or physical clogging of the process machinery. The criteria for selecting one technology upon another is not always experimentally or rationally driven, and in many cases such decisions are made due to the familiarity with, and availability of, one of these technologies in the research lab or pilot plant. Ultrasound has been used as a tool for controlling particle size by the pharmaceutical industry for many years (10). The fact that ultrasonic waves can induce a wide range of mechanical effects in liquids makes them attractive to an industry that depends greatly on liquid-phase reactions. In addition, post reaction processing, to reduce particle size, is a common step in the preparation of active pharmaceutical ingredients (API). Particles can be formed, for example, of a drug crystalline or, alternatively, from a liposome that encapsulates the anticancer agent doxorubicin.

NEXT GENERATION ULTRASOUND SYSTEMS

Ultrasound technologies have made great progress over the past years. The traditional ultrasonic probe or bath, have been replaced by accurate computer-controlled systems. The main driving force for this technological advancement was the introduction of new clinical tools, such as high intensity focused ultrasound (HIFU) (11), which is used for tumor and fibroid ablation. Needs posed by molecular biologists for research

tools that can shear or extract DNA without damaging delicate biomacromolecules triggered the invention of bench-top focused-ultrasound chambers (12). These ultrasonic tools are now being adapted for pharmaceutical formulation. Ultrasound is defined as oscillating acoustic pressure waves having wavelengths (λ) above the audible threshold, i.e. greater than 18 kHz. High frequency ultrasound (λ > 3 MHz), is widely used for medical imaging. Midrange ultrasound, i.e., λ = 1-3 MHz, is used in the clinic for 'gentle' procedures, such as physiotherapy and soft tissue relief. Low frequency ultrasound (18 kHz -1 MHz) is mainly used in the clinic for procedures that transiently or permanently affect tissue, such as kidney stone shattering, tumor ablation, ocular surgeries, and increasing skin permeability to enable needleless transdermal drug delivery (2, 13). Ultrasonic waves can be tuned to be of increasing amplitudes, thereby addressing various clinical/experimental needs.

THE MECHANISMS BY WHICH ULTRASOUND AFFECTS MATTER

The mechanism by which ultrasound interacts with matter can be divided into three categories: cavitation, shear, and collision. Cavitation (also known as transient cavitation) is the growth and implosion of gas bubbles in a solution. The ultrasonically-induced oscillating pressure field drives cavitation, especially at lower frequencies (14) or when a low frequency is combined with high frequency ultrasound (15). The implosion of the cavitating bubble creates a forceful jet with a calculated pressure impact of more than 1000 atmospheres and a rapid adiabatic heating and cooling rate of 10 billion Kelvin per second (16) as well as high local shear rates. The cavitation jet acts as a jackhammer, rupturing crystalline materials or porating soft materials (17). Therefore, cavitation is highly efficient for downsizing particles (Figure 1). However, cavitation may also harm delicate compounds in the formulation (18). Therefore, a preliminary study must be conducted to evaluate the physicochemical stability of the irradiated compounds under different ultrasonic amplitudes. Similarly, a graph correlating the ultrasonic amplitude, irradiation time and resultant particle size, can be derived. Next-generation formulation systems will be equipped with analytical capabilities and programed to measure parameters such as size, chemical integrity and configuration at real time, thereby enabling high throughput rapid formulation. Ultrasound also induces intense mixing effects. Ultrasonic mixing is generated by the physical vibrations of an ultrasonic probe that comes in contact with a liquid, and/or by acoustic streaming (liquid motion that is driven by the attenuation of a propagating ultrasonic beam). While the velocity of sound waves is nearly 1480 m/s in water, the velocity of particles streaming under the ultrasonic field is far slower, usually below 1 m/s (19-23). The momentum induced by such velocities seems insufficient for downsizing microparticles by inter-particular collisions (24). However, these streaming effects are efficacious for homogeneously dispersing powders in liquids or for forming

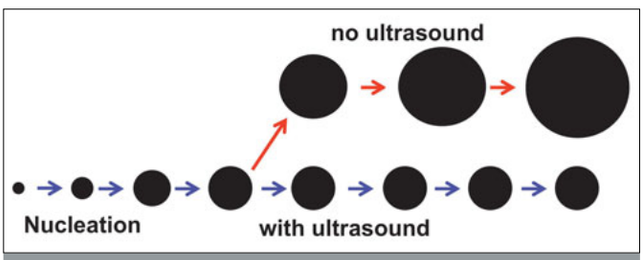

Figure 2. Ultrasound can be used for controlling the growth of crystals in solution by homogenously dispersing the precipitating material.

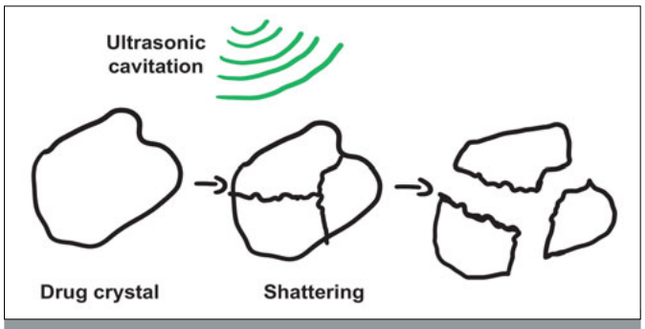

Figure 1. Ultrasonically-induced transient cavitation, i.e., the growth and implosion of gas bubbles, creates a forceful jet and intense shearing of particles in its vicinity; this phenomenon is widely used for downsizing drug crystals.

micelles/liposomes loaded with hydrophobic drugs (10). In addition, these mixing effects have been shown by us and others to control crystal growth rates, by homogeneously dispersing the crystalizing material among all the nucleation seeds (Figure 2) (25-27). Ultrasonic mixing is effective for controlling processes while they occur (such as crystal growth or dispersing solids in liquids), however, once a process is complete (post crystal or liposome formation) higher power magnitudes, such as cavitation, are necessary for reducing particle size.

ULTRASOUND CONCENTRATES PARTICLES IN SOLUTION

Ultrasound holds great promise for nano and micro post-formulation processes, specifically, for separating and concentrating particulate sub populations (28, 29).

In 1926, Albert Einstein explained why herb leaves in a stirred cup of tea are propelled to the center of the cup, rather than migrating to the cup's outer perimeter by centrifugal forces (30). He showed how an increasing radial pressure gradient is formed in the stirred liquid, and how it affects the leaves captured in the cup's core. These principles were then adapted, using standing ultrasonic waves, to concentrate particles dispersed in a solution (31-34). Separating particle' sub populations according to size and structure, or concentrating them, remain great challenges in the field of drug delivery. Despite the fact that ultrasound-facilitated separation and concentrators has been studied extensively in academia, a commercially available bench top ultrasound concentrator/separator has yet to be developed. Such a system will address pressing needs in translational laboratories and API industries.

ULTRASOUND-RESPONSIVE DRUG DELIVERY SYSTEMS

Next-generation pharmaceutical formulation will not end in the production plant or pharmacy. We are entering an era in which the therapeutic activity of a formulation will greatly depend on, and be tuned to, respond to external stimuli. Ultrasound is playing an important role in realizing these drug/device combinations. For example, ultrasound has been used by us and others to trigger drug release from nano carriers inside tumors. Focusing drug release within the diseased tissue proved to be an improved treatment modality in comparison to current, non-targeted, practice. In order to respond to ultrasound the nano carrier must be engineered at the molecular level. Liposomes with polyethylene glycol embedded in their lipid bilayer were found to be more responsive to ultrasound, while cholesterol-rich liposomes are less ultrasound responsive. To improve the intra-cellular uptake of therapeutics several groups have conjugated gas-filled microbubbles (such as perfluorocarbons) to a drug. In the vicinity of the diseased cells an oscillating ultrasonic field is applied to implode the bubbles, thereby creating a forceful jet that drives the drug into the cells. This strategy has been used to deliver a wide variety of therapeutics, such as chemotherapies, nucleic acids and even nanoparticles.

CONCLUSIONS

In summary, ultrasound technologies began their mass development during World War I, but since then these technologies have been used mainly for the benefit of humankind (35). Miniature ultrasonic devices, such as ultrasonic catheters, patches and even swallowable ultrasonic pills, are being used to address a wide scope of medical needs. In tandem, ultrasonic technologies are being used for improving pharmaceutical formulation by controlling the particle size of crystals, liposomes, and micelles. Future applications will include ultrasonic-based biomacromolecule formulation as well as ultrasonic-based particle sorting and concentrating. The rather simple ability to control ultrasonic wavelength, amplitude and spatial dispersion, as well as commercial availability of off-the-shelf ultrasonic components makes ultrasound an attractive tool for addressing the challenging needs of advanced pharmaceutical formulation.

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