Exploitation of Acoustic Cavitation-Induced Microstreaming to Enhance Molecular Transport

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ABSTRACT: Ultrasound (US) exposure of soft tissues, such as the skin, has been shown to increase permeability, enhancing the passage of drug molecules via passive processes such as diffusion. However, US regimes have not been exploited to enhance active convective transport of drug molecules from a donor layer, such as a gel, into another medium. A layered tissue-mimicking material (TMM) was used as a model for a drug donor layer and underlying soft tissue to test penetration of agents in response to a range of US parameters. Influence of agent molecular mass (3–2000 kDa), US frequency (0.256/1.1 MHz) and US pressure (0–10 MPa) on transport was characterised. Agents of four different molecular sizes were embedded within the TMM with or without cavitation nuclei (CN) and US applied to achieve inertial cavitation. Post-insonation, samples were analysed to determine the concentration and penetration distance of agent transported. US exposure substantially enhanced transport. At both US frequencies, enhancement of transport was significantly higher ($p < 0.05$) above the cavitation threshold, and CN reduced the pressure at which cavitation, and therefore transport, was achieved. Acoustic cavitation activity and related phenomena was the predominant transport mechanism, and addition of CN significantly enhanced transport within a range of clinically applicable acoustic pressures. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1903–1912, 2014

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INTRODUCTION

Non-invasive drug and vaccine delivery is garnering a large amount of research interest because of the substantial risk associated with the delivery of drugs and vaccines via needle and syringe.[1] In response, a wide range of needle-free delivery techniques have been developed, such as ocular delivery via iontophoresis and transdermal delivery via jet injection or sonophoresis.[2] Many of these approaches benefit from improved safety and increased patient compliance[3] as well as potentially enabling self-administration and sustained release.[4]

Barriers to non-invasive delivery often exist as layers of tissue that function as barriers themselves, with very low permeability to foreign molecules. These layers [such as the stratum corneum (SC) of the skin] require permeabilisation by chemical or mechanical means to allow the penetration of drug and vaccine molecules. A number of strategies have been deployed to increase the permeability of soft tissues such as the skin to molecules of more than 500 Da, including agent formulation optimisation,[5] application of minimally invasive delivery (microneedles and jet injections)[6] or the use of energy-driven methods.[7] Previous work has shown ultrasound (US) to be capable of skin permeabilisation by exploitation of the use of ultrasonic waves.[8–10] The numerous studies devoted to understanding the mechanisms of sonophoresis, and in particular how different US parameters affect the permeability of skin have identified two broad mechanistic categories: (1) thermal and (2) non-thermal.

Thermal mechanisms have been implicated in the enhanced molecular transport observed after or during US exposure. Transfer of thermal energy to the drug molecules themselves enhances their diffusion through a barrier, particularly if the barrier has already been permeabilised.[11] However, depending on the exposure conditions, this mechanism can become detrimental to drug delivery as prolonged heating damages both tissue and drug.

Mechanical pheonomena, and particularly acoustic cavitation, therefore play the dominant role in enhancing drug delivery by US. Stable cavitation refers to the periodic oscillation of bubbles over several acoustic cycles, whereas inertial cavitation is the dramatic expansion of bubbles during the rarefactional ultrasonic half-cycle, followed by their violent collapse during the compressional half-cycle under the effect of the inertia of the surrounding medium.[12] The *inertial cavitation threshold* corresponds to the minimum acoustic pressure amplitude required to induce rapid growth and violent collapse of cavitation bubbles. The growth of cavitation bubbles becomes increasingly difficult with increasing US frequency because of the reduced timescale available for bubble growth, and as a consequence, the threshold pressure for occurrence of inertial cavitation is proportional to US frequency.[13] Inertial cavitation can be identified by the broadband acoustic response it generates, whereas stable cavitation can be identified by the harmonics and subharmonics of the fundamental US frequency it produces.[12] It is therefore possible to remotely map these cavitation events and characterise their role in the extent and location of delivery.[14]

The predominant mechanisms by which permeability of a barrier layer is increased are believed to be (1) acoustic streaming, arising from attenuation of US as it propagates through a medium, and (2) microstreaming resulting directly from cavitation activity of gas bubbles within a medium.[15] Furthermore, acoustic radiation force can also act upon drug or vaccine particles if they are of comparable dimensions to, or larger than, the wavelength of the US beam, pushing them through a medium and thereby increasing molecular penetration.[16] Such radiation forces will not be significant when using nano- or micro-sized

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particles in a kHz US field, but will begin to dominate in the presence of MHz-range US.[17]

Using the example of transdermal drug delivery, most studies exploring the use of sonophoresis have introduced a drug to the skin following pre-treatment with US to increase permeability,[18] leading to clinically approved products such as the SonoPrep device (Echo Therapeutics, Philadelphia, PA, USA), used to deliver local anaesthesia via the transdermal route.[19] These studies have avoided the simultaneous application of the drug and US because of the complications that arise from having to make a drug formulation that is stable to US exposure. However, because the drug or vaccine is not exposed to US during delivery, ultrasonic mechanisms such as microstreaming and acoustic radiation force are not exploited to increase the *transport* of the agent across the medium.

In the present study, it is hypothesised that if a stable drug/vaccine formulation can withstand US exposure, delivery will be more effective. Increased effectiveness is expected to arise not only from the increased barrier permeability due to sonophoresis, but also because of the effects of acoustic cavitation and acoustic radiation force present in the US field that will increase propulsion of the vaccine molecule through a permeabilised membrane.[20] There is already evidence in the literature supporting this hypothesis.[3,21,22] In these studies, however, the drug was delivered in solution, which is not clinically practical.

The present study therefore proposes the integration of the vaccine within an already clinically approved coupling gel. Coupling media (e.g., hydrogels) can be placed between the US transducer and the skin to provide a regulated source of cavitation[23] and a medium through which to transmit the US energy from the transducer into skin. Since the permeability of the barrier membrane will be increased through cavitation, and the vaccine molecules actively transported, US gels can be optimised to enhance the quantity and quality of drug delivery. Previous studies in the field of transdermal hydrogels in particular have focussed on enhancing drug absorption via a cutaneous route by introducing chemical absorption enhancers such as sodium lauryl sulphate[21] or incorporating a drug into a hydrogel and relying on passive diffusion for skin uptake.[24] Both methods have substantial disadvantages, as chemical absorption enhancers result in skin damage, and passive diffusion is a slow process limited to small, hydrophobic molecules.

Although there is a notable amount of literature in the field of US -assisted transdermal drug and vaccine delivery, we believe that this study is the first to examine the mechanism by which *molecular transport* (as opposed to increased permeability) is affected by US. As this work represents a mechanistic study, the tissue-mimicking material (TMM) used was designed to replicate the pore size and structure of permeabilised skin as an example of soft tissue through which molecular transport could occur. Actual skin was not used because issues of sample-to-sample variability from excised animal or human skin, which notoriously vary in hydration and conductivity, would prevent identification and optimisation of the underlying mechanism. A layered agarose TMM was therefore used as a model for the donor layer, barrier membrane and recipient layer to test the penetration of agents in response to a range of US parameters. In particular, the influence of agent molecular mass (over a 3–2000 kDa range), US frequency (0.256 or 1.1 MHz) and US pressure (over a 0–10 MPa range) on transport was characterised. After inertial cavitation thresholds of the TMM were determined, agents of four different molecular sizes were embedded within the TMM in the presence or absence of cavitation nuclei (CN) and US applied to achieve no cavitation or inertial cavitation. Following insonation, samples were analysed to determine the concentration and penetration distance of molecular agent transported for each set of US parameters, enabling identification and optimisation of the key underlying mechanisms.

The present work aims to quantitatively and mechanistically study *in vitro* the optimal mechanism by which US-assisted active transport of a range of sizes of drug and vaccine molecules can be achieved.

MATERIALS AND METHODS

A TMM was formulated, and cavitation events within this model during US exposure were monitored to determine its inertial cavitation threshold. Samples of the TMM were then exposed to different US frequencies and pressures, selected to be both below and above the cavitation threshold at each frequency. Cavitation activity was measured in all experimentation using a passive cavitation detector (PCD). Post insonation, samples were quantitatively analysed using fluorescence microscopy to visualise different sized molecules to determine the amount and distance of model drug transported, with the aim of determination of the dominant mechanisms of transport of molecules within the gel. The effect of the addition of CN on transport was also investigated to determine their potential usefulness in enhancing transport.

Tissue-Mimicking Material

Agarose gel has been widely used as a TMM.[25] Building on previous studies, a concentration of 0.5% (w/v) was used to mimic both the US gel and the soft tissue delivery target. The structure and porosity of agarose gel at this concentration is close to that of soft tissues such as the skin (agar pore size range 0.5–50 μm,[26] skin pore size 0–30 μm diameter).[27] Cylindrical samples of degassed 0.5% (w/v) agar (UltraPure Agarose 1000; Life Technologies, Paisley, UK) were prepared by dissolving the powdered agar in distilled, deionised water and heating to 90°C until all the powder had dissolved. The agar was then degassed at 50°C and pipetted while warm and liquid into cylindrical moulds (30 mm length × 10 mm cross-sectional diameter). This size of sample was chosen because of the size of the focus of the US beam, and to allow efficient use of fluorescently labelled dextrans. Four differently sized and labelled dextrans were incorporated into an additional layer of gel (200 μL agar/dextran/talc solution), which was applied over one cross-sectional surface of the previously gelled cylinder (Fig. 1): 3 kDa dextran labelled with Cascade Blue Dye (Molecular Probes D7132; Life Technologies, Paisley, UK), 70 kDa dextran labelled with Cy5 dye (Dex0013–4; Nanocs, New York, NY, USA), 155 kDa dextran labelled with TRITC (T1287; Sigma–Aldrich, Gillingham, UK) and 2000 kDa dextran labelled with FITC (FD20S; Sigma–Aldrich, Gillingham, UK), at concentrations selected for optimal fluorescence visualisation (80 μg/mL for 3, 70 and 2000 kDa molecules and 160 μg/mL for 155 kDa molecules). Dextran masses represented a range of molecular sizes corresponding to the sizes of the drugs and vaccines in clinical use. Dextran molecules were chosen for use because, although their charge and branching characteristics differ from many conventional