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Relations between acoustic cavitation and skin resistance during intermediate- and high-frequency sonophoresis

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ABSTRACT

Enhanced skin permeability is known to be achieved during sonophoresis due to ultrasound-induced cavitation. However, the mechanistic role of cavitation during sonophoresis has been extensively investigated only for low-frequency (LFS, <100 kHz) applications. Here, mechanisms of permeability-enhancing stable and inertial cavitation were investigated by passively monitoring subharmonic and broadband emissions arising from cavitation isolated within or external to porcine skin *in vitro* during intermediate- (IFS, 100–700 kHz) and high-frequency sonophoresis (HFS, >1 MHz). The electrical resistance of skin, a surrogate measure of the permeability of skin to a variety of compounds, was measured to quantify the reduction and subsequent recovery of the skin barrier during and after exposure to pulsed (1 second pulse, 20% duty cycle) 0.41 and 2.0 MHz ultrasound over a range of acoustic powers (0–21.7 W) for 30 min. During IFS, significant skin resistance reductions and acoustic emissions from cavitation were measured exclusively when cavitation was isolated outside of the skin. Time-dependent skin resistance reductions measured during IFS correlated significantly with subharmonic and broadband emission levels. During HFS, significant skin resistance reductions were accompanied by significant acoustic emissions from cavitation measured during trials that isolated cavitation activity either outside of skin or within skin. Time-dependent skin resistance reductions measured during HFS correlated significantly greater with subharmonic than with broadband emission levels. The reduction of the skin barrier due to sonophoresis was reversible in all trials; however, effects incurred during IFS recovered more slowly and persisted over a longer period of time than HFS. These results quantitatively demonstrate the significance of cavitation during sonophoresis and suggest that the mechanisms and post-treatment longevity of permeability enhancement due to IFS and HFS treatments are different.

1. Introduction

The skin offers a convenient and easily accessible route of drug administration for local and systemic treatment with the advantages of avoiding pre-systemic absorption and systemic toxicity while also providing sustained, rate-controlled delivery [1]. However, the prevalent use of the transcutaneous route is severely limited due to the robust permeability barrier presented by the highly ordered lipid bilayer structure of the outermost skin layer, the stratum corneum (SC). A variety of non-invasive technologies have therefore been investigated with the aim of increasing skin permeability by transiently perturbing the SC architecture. Among these technologies, the application of therapeutic ultrasound to skin, a treatment termed sonophoresis, has proven to be particularly promising.

The primary enhancement mechanism of sonophoresis is believed to be acoustic cavitation, a phenomenon that has been widely researched in a number of other ultrasound-enhanced drug delivery applications [2,3]. Specifically, enhanced drug delivery has been shown to be achieved when cavitation interacts with and modifies the permeability of biological interfaces such as the blood brain barrier [4], fibrin matrix of blood clots [5,6], and plasma membrane of cells [7]. The mechanisms of permeability enhancement among these applications are typically dependent on physical effects produced by distinct dynamic responses of a bubble to ultrasound, which are broadly categorized as either stable or inertial. Stable cavitation is characterized by repetitive, low-amplitude bubble oscillations which produce subharmonic emissions [8,9] and high-velocity microstreams around the oscillatory boundary layer of the bubble [10]. Inertial cavitation refers to the rapid expansion of a microbubble followed by a violent collapse, leading to microjet [11] and shock wave [12] formations as well as generating

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broadband noise due to the supersonic acceleration of the bubble wall during collapse [9].

In the context of sonophoresis, increased skin permeability can be achieved when cavitation bubbles interact with and modify the skin barrier by inducing, dilating, and connecting defects [13–15] to form regions of increased permeability within the SC [16,17]. However, the precise mechanisms, specifically the location(s) and type(s) of cavitation, responsible for skin permeabilization may be dictated by the frequency of ultrasound used for sonophoresis. In a study designed to identify the location of permeability-enhancing cavitation during low-frequency sonophoresis (LFS, <100 kHz), skin electrical resistance, used as a surrogate measure of skin permeability, was shown to be significantly reduced only when cavitation was present within the donor medium outside of skin [18]. Moreover, this study demonstrated that cavitation within the skin was unlikely to occur or provide a significant effect on skin permeability during LFS. Investigations aimed at identifying the critical types of permeability-enhancing cavitation during LFS have demonstrated a strong correlation between enhancement and measured broadband acoustic emissions, indicating that inertial cavitation occurring outside of the skin is the primary permeabilization mechanism of LFS [18–20]. Furthermore, theoretical [21] and experimental [22] investigations have indicated that the permeabilization effect from inertial cavitation during LFS is primarily due to microjet formations, manifested during the asymmetric collapse of bubbles near the skin surface, that impact and locally perturb the SC. Exploitation of this well characterized mechanism has led LFS treatments to provide a greater, although reversible, permeabilization effect while also significantly reducing treatment times [23,24].

Since higher ultrasound frequencies result in cavitation bubbles with smaller dimensions and different dynamics than those produced by lower frequencies [25], the role of cavitation during intermediate- (IFS, 100–1000 kHz) and high-frequency sonophoresis (HFS, >1 MHz) is likely different from during LFS. Among studies investigating IFS, Wu et al. identified the existence of randomly-arranged, air-filled voids approximately 20 μm in diameter within the SC of human skin, apparently due to cavitation, after *in vitro* exposure to 168-kHz ultrasound [13]. In another study, increased enhancement of skin permeability was shown by Ueda et al. to scale directly with rising broadband emissions, emanating from inertial cavitation presumed to be occurring within the donor medium outside the skin, during sonophoresis using frequencies as high as 445 kHz [19].

Unlike LFS and possibly IFS, cavitation within the skin may play a greater role during HFS because at higher frequencies resonant bubble diameters are smaller, comparable to dimensions of the lacunar voids within the skin where cavitation can occur [26–28]. This assertion was investigated experimentally in a study by Mitragotri et al., showing that significant changes in skin electrical resistance occurred when the potential for cavitation was isolated to within the skin [15]. Microscopy-based analysis of skin after HFS in this study identified a disarrangement of the SC lipid bilayer, further indicating that skin permeabilization was due to cavitation occurring within voids near the corneocytes of the SC. In a study by Park et al., penetration of fluorescein isothiocyanate (FITC)–dextrans across skin during HFS was significantly enhanced when cavitation nuclei, in the form of ultrasound contrast agents (UCA), were introduced to the surrounding medium [29]. Due to the relatively low pressures used for HFS in this study, the observed enhancement was suggested by these authors to have been caused by microstreaming associated with stable cavitation occurring outside the skin.

Although cavitation has been widely accepted as the primary enhancement mechanism of sonophoresis, the effects on skin permeability due to the various location(s) and type(s) of cavitation that occur during IFS and HFS, which are likely different from those of LFS, have not been fully elucidated. The conclusions of previous IFS and HFS investigations have been based on theoretically-derived results or indirect observations and have yet to be investigated systematically and quantitatively. This lack of mechanistic understanding has therefore inhibited optimization efforts to exploit and control specific permeability-enhancing mechanisms of cavitation necessary to improve treatment efficacy of IFS and HFS. Moreover, although sonophoresis using higher frequency ultrasound has a long track record of safety [30], the reversibility and longevity of enhanced permeability due to IFS and HFS is not well characterized.

Here, mechanisms of cavitation were quantitatively investigated by passively measuring acoustic emissions associated with specific cavitation activity isolated within or outside porcine skin during *in vitro* IFS and HFS experiments. Since the correlation between the electrical impedance and permeability of skin has been quantitatively defined for a variety of compounds, including hydrophilic and hydrophobic solutes [31–33], skin resistance can be used to instantaneously monitor alterations made to the skin barrier during sonophoresis as a surrogate measure of permeability [15,18,20] and to monitor the barrier recovery [34–36]. Resistance measurements of porcine skin were made here as an indicator of time-dependent changes in skin permeability during and after sonophoresis. Measured emission levels and skin resistance values were compared to clarify the potential roles of cavitation during IFS and HFS treatments, including (1) permeabilization associated with cavitation activity inside or outside the skin, (2) the relationship of emissions from distinct cavitation type(s) with treatment efficacy metrics, such as faster and greater overall permeabilization of skin, and (3) the reversibility and longevity of perturbations made to the skin due to ultrasound treatments in these frequency regimes.

2. Materials and methods

2.1. Skin tissue preparation

Fresh skin was harvested from the front lateral flank of female Yorkshire X swine immediately *post mortem* under the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Cincinnati. Full thickness skin (FTS) samples were prepared by removing subcutaneous tissue and excess hair, sectioned into 6 × 6 cm square pieces, and stored at −80 °C until use within a period of less than 3 months, to avoid altering the barrier due to storage [37,38]. Experiments were conducted using *in vitro* porcine skin since previous studies have shown, utilizing skin electrical resistance as an indicator of skin permeabilization, that porcine skin may provide similar drug transport pathways as human skin *in vitro* [33] and that *in vivo* permeability may be accurately predicted from *in vitro* measurements of porcine skin [16]. Additionally, porcine skin was used due to its comparable histological, biochemical, and *in vitro* permeability characteristics to human skin [39,40].

Prior to use, the FTS samples were thawed and hydrated in 0.01 M phosphate buffered saline (PBS; Sigma-Aldrich, St. Louis, MO USA) at 4 °C for 20–24 h to eliminate temporal changes of skin permeability due to a hydration gradient during experiments. The gas content of PBS used for FTS sample hydration and experiments was monitored using a meter (WD-35641, Oakton Instruments, Vernon Hills, IL USA) to measure dissolved oxygen (DO) as a surrogate measure of air saturation. The DO content of skin was controlled by hydrating skin using PBS that was either: (1) degassed by placing under vacuum while in a sonication bath for approximately one hour to lower the DO below 20%, or (2) allowed to saturate in open air, permitting the gas content to reach a DO of at least 80%. After hydration and immediately prior to use, skin was acclimated to room temperature for 30 min in fresh PBS that was approximately the same DO as used during hydration.

2.2. Ultrasound apparatus and in vitro experiments

Hydrated FTS samples were trimmed into circular sections 4.5 cm in diameter and placed in a custom made vertical diffusion cell, separating PBS filled donor and receiver compartments of 3.1 cm diameter, with