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Quantitative Evaluation of the Sonophoresis Efficiency for Large Nanoparticles

Sonophoresis efficiency for large nanoparticles

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Abstract— **Transdermal drug delivery has a critical contribution to medical practice and some advantages over conventional oral administration and hypodermic injection. Enhancement of percutaneous absorption or penetration of a therapeutic agent by ultrasound (sonophoresis) has been applied and studied for decades. In this study, the penetration percentage through the porcine ear skin specimen was determined quantitatively by measuring the fluorescence from nanoparticles in the size of 60 nm, 220 nm, and 840 nm in the receptor chamber at different sonication parameters. It is found that the sonophoresis efficiency increased with the acoustic intensity, duty cycle, and sonication duration, but decreased with the particle size (62.6±5.4% for 60 nm vs. 11.9±1.1% for 840 nm polystyrene nanospheres after 30 min sonication at 0.5 W/cm^2 and 100% duty cycle, $p < 0.05$). Although the pore size kept almost the same in scanning electron microscopy, more flakes were observed at the entry site with the progress of sonication. In summary, sonophoresis efficiency is dependent on the ultrasound parameters and particle size and satisfactory penetration of even sub-micro objects through the pores is feasible.**

Keywords—transdermal drug delivery, sonophoresis, fluorescent nanoparticles, quantitative drug penetration, cavitation

I. Introduction

Oral administration and hypodermic injection are routine approaches of drug delivery. However, they are associated with the premature drug metabolization by the first-pass effect of the liver and gut wall, the degradation of drugs by the digestive tract, unstable plasma level, difficulty in immediate termination, painfulness, dangerous medical waste, and the risk of disease transmission by needle re-use (Prausnitz and Langer 2008). Transdermal drug delivery (TDD), an noninvasive alternative, can be self-administered, long-released, and inexpensive. The development of TDD includes chemical enhancer, iontophoresis, biochemical enhancers, electroporation, and micro-needles. The skin structure and elasticity can be altered to enable increased permeation of compounds temporarily and resumed a few hours after the termination without permanent damage. However, only a limited number of drugs with molecular weights up to only a few hundred Daltons (Da) are amenable for the treatment of local allergies or infections. Delivery of peptides, hydrophilic drugs, and macromolecules (i.e., DNA or SiRNA in genetic therapy) is still challenging. Therefore, TDD occupies only 10% of the entire market of drug delivery (Brown 2002).

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Ultrasound has been used in TDD since the 1950s, termed as sonophoresis or phonophoresis (Byl 1995, Mitragotri et al. 1995), and can disrupt lipid structure in the stratum corneum (SC) and thereby increase permeability utilizing either noncavitation or cavitation effects. Noncavitation ultrasound is generally limited to enhancing small lipophilic compounds in TDD while cavitation one increases skin permeability for up to many hours without damaging deeper tissues. Sonophoresis is generally safe with no negative short- or long-term side effects, including tissue heating, and has been approved by the United States Food and Drug Administration (USFDA) for lidocaine and studied extensively for insulin, heparin, tetanus toxoid vaccine (Prausnitz and Langer 2008, Polat et al. 2011).

To maximize therapeutic efficacy, several new drug carriers have been investigated. Liposomes are bicompatible and biodegradable vesicles in diameter of about 25-10,000 nm with encapulation of both hydrophilic and lipophilic drugs. Ethosomes enable drugs to reach the deep skin layers and/or the systemic circulation (Godin and Touitou 2003). Microparticles and nanoparticles can incorporate drugs or antigens in the form of solid solutions or dispersions and have been shown to enhance the delivery of certain drugs across a number of natural and artificial membranes. Metal nanoparticles are recognized for seemingly small drug-like characteristics, i.e. antimicrobial activity and skin cancer prevention. However, nanoparticles > 10 nm in diameter are unlikely to penetrate through the SC into viable human skin but will accumulate in the hair follicle openings (Prow et al. 2011). Biodegradable nanoparticles (150-250 nm) have been used for transdermal DNA delivery using a low-pressure gene gun (Lee et al. 2008).

Although the effectiveness of sonophoresis has been demonstrated, effect of ultrasound parameters (frequency, acoustic intensity, duty cycle and exposure duration) on the outcome is not clear, and there are few studies of large size drug carrier in sonophoresis. In this study, the drug penetration efficiency was quantified by measuring the fluorescence of nanoparticles (60 nm, 220 nm, and 840 nm) in the receptor chamber through the porcine ear skin specimens with varied acoustic intensity (0.3-1 W/cm^2), duty cycle (20-100%), and duration (7-30 minutes). Sonicated samples were observed with a scanning electron microscope for cavitation-induced damage. It is found that owing to the hair removal the sonophoresis has a much higher penetration efficiency, even for sub-micron particles, which increases with the acoustic intensity, duty cycle and sonication duration but decreases with the particle size.

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II. Materials and Methods

Porcine ear skin was used here because of its similar dermatopharmacokinetic characteristics (i.e., diffusion and partition of the drug in the SC) as human skin and prepared using the previously described protocols (Sekkat et al. 2002, Herkenne et al. 2006). Fresh porcine ears were purchased from a local slaughterhouse (Primary Industrial Pte Ltd, Singapore) after the approval from Agri-Food Veterinary Authority (AVA) of Singapore, cleaned under cold running water, and immersed in the phosphate buffered saline (PBS) solution to remove remaining lipids. Any visible hairs were carefully plucked out. Skin membranes were removed from the underlying cartilage, cut into the size of 2.3×2.3 cm, wrapped in aluminium foil, and then stored at -20°C for use within three months. Thickness of skin specimens were 1.5±0.4 mm measured by a digital caliper. On the day of the experiment, the tissue samples were soaked in PBS at room temperature (23 ± 1°C) for about 1 hour to thaw the specimen and to allow its initial barrier property to reach a steady state.

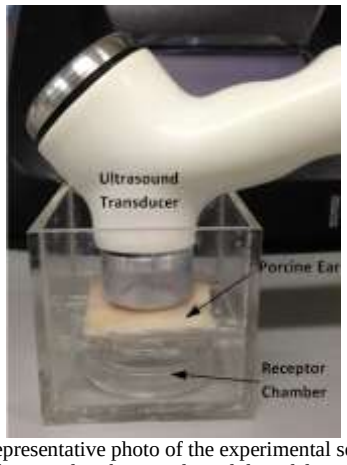

Figure 1. Representative photo of the experimental setup for using ultrasound in the transdermal drug delivery.

The skin membranes were mounted with the SC towards the ultrasound transducer. Sky Blue dyed polystyrene nanoparticles in the size of 60 nm, 220 nm and 840 nm (Spherotech, Lake Forest, IL) were used because of their emission and excitation wavelength matching the fluorescent detector module. 30 μL of nanoparticles was spread evenly across the specimen. Then an ultrasound transducer (Therasound Evo, Rich-Mar, Chattanooga, TN) with the driving frequency of 1 MHz, spatial-average temporal-peak intensity up to 2 W/cm^2, varied duty cycle (DC) from 20% to 100% (continuous wave), and a diameter of 2 cm was applied onto it. Before each experiment, the transducer was cleaned by distilled water thrice to remove the remaining particles. A receptor chamber filled with 2.6 mL of distilled water was underneath the skin sample and on the top of a piece of rubber sheet with thickness of about 5 mm to reduce the propensity of generating standing waves. The experimental setup is shown in Fig. 1.

After the sonication, the solution in the receptor was mixed homogeneously, and then 1.3 mL solution was extracted to measure its fluorescent standard unit (FSU) by a fluorometer (GloMax®-Multi Jr, Promega, Madison, WI) using a green fluorescence optical kit with an excitation wavelength of 525 nm and emission wavelength of 580-640 nm. FSUs of varied concentrations of nanoparticles in the distilled water (30 μL in 2.6 mL distilled water as 100%) were measured to draw a calibration curve, from which the concentration of nanoparticles in the receptor chamber and the corresponding penetration efficiency could be determined quantitatively.

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Skin specimen was then washed with PBS solution thrice and fixed by 2.5% glutaraldehyde (Sigma-Aldrich) overnight at 4 ºC. Samples were washed next day with PBS to remove glutaraldehyde, dehydrated with increasing concentration of ethanol from 25% to 100%, dried using a critical point dryer (CPD 030, Bal-Tec, Witten/Ruhr, Germany), and then photographed using an SEM (JSM-5600LV, JEOL, Tokyo, Japan) with an appropriate magnification at different positions of the sonicated region to observe the morphological changes.

A two-way analysis of variance (ANOVA) (two-factor without replication, $p = 0.05$) was performed to test the statistical significance between the treatment protocols using SPSS Statistics (IBM Software, Somers, NY). The sample size in each testing condition was at least five, and all samples were completely independent.

III. Results

FSUs of varied nanoparticle concentrations, from 10% to 100%, can be fitted quite well by a straight line with the zero-bias as the error of the fluorescence detector. The variation is usually less than 0.3% except at the concentration of 100%, which may be due to the interferences among concentrated nanoparticles. Sonication at the intensity of 1 W/cm^2 and DC of 100% or immersing in a water bath at the temperature of 40 °C for 30 minutes will not change the calibration results significantly.

TABLE I. Percentage of 220 nm nanoparticle penetration after 7-minute sonication at the varied duty cycle and acoustic intensities

	0.3 W/cm^2	0.5 W/cm^2	1 W/cm^2
20% DC	2.7±0.7%	2.9±0.6%	3.5±0.8%
50% DC	4.0±1.1%	4.4±1.0%	6.1±1.3%
100% DC	5.4±1.4%	8.1±1.6%	10.2±1.9%

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DC: duty cycle

The penetration percentages of 220 nm nanoparticles after the 7-minute sonication at the varied acoustic intensity and DC are listed in Table 1. It is found that with the increase of acoustic intensity from 0.3 to 1.0 W/cm^2 and DC from 20% to 100% the drug penetration efficiency increased from 2.7±0.7% to 10.2±1.9%. The results of 60 nm, 220 nm, 840 nm nanoparticles after 7-, 15-, and 30-minute sonication at the acoustic intensity of 0.5 W/cm^2 and DC of 100% are shown in Fig. 3. The dependencies on the particle-size and sonication duration are apparent. For 60 nm nanoparticles, the transdermal penetration efficiency increases from 28.0±2.3% at 7-minute to 62.6±5.4% at 30-minute sonication. For even sub-micro particles (840 nm, whose molecular weight is about 1.69×10^{11} Da) [18], satisfactory results can also be obtained after the 30-minute sonication (11.9±1.1%). In addition, the temperature elevations in all conditions were less than 2°C as measured by a thermocouple and a thermometer (Omega, Stamford, CT, USA), which shows the thermal effect is not the major mechanism for the enhanced penetration.

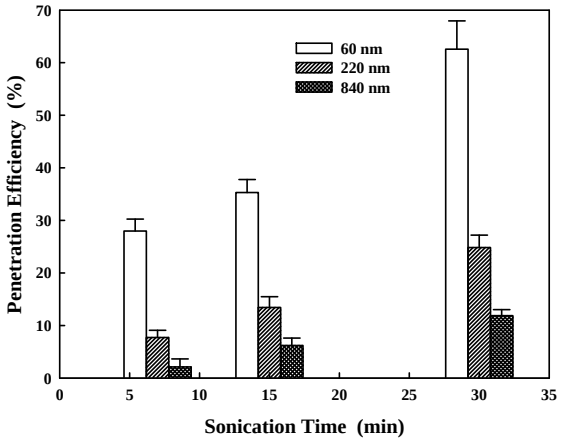

Figure 2. Transdermal drug penetration efficiencies using ultrasound at the acoustic intensity of 0.5 W/cm^2 for 60, 220, and 840 nm fluorescent nanoparticles after sonication of 7, 15, and 30 minutes.

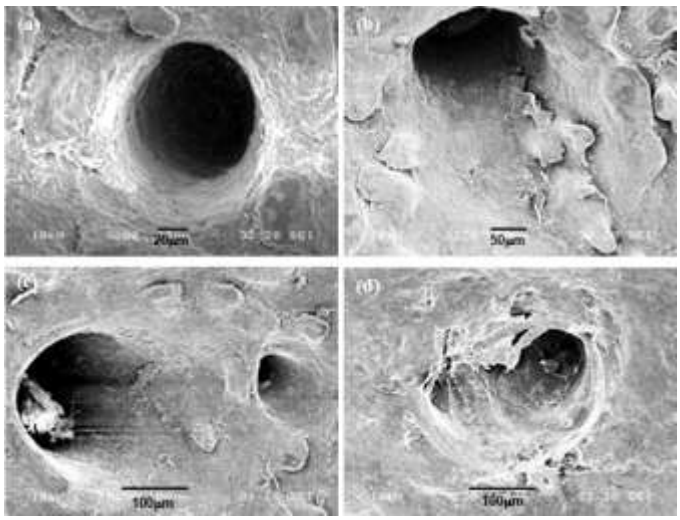

Figure 3. Representative surface electron microscope (SEM) photos of (a) porcine skin samples, and the tissue samples after sonication of (b) 7 mins, (c) 15 mins, and (d) 30 mins at the acoustic intensity of 0.5 W/cm^2 with 100% DC (continuous wave).

SEM images of specimen after 7-minute sonication at the acoustic intensity of 0.5 W/cm^2 did not show damage on the skin, but a little flaking occurred inside and around the skin pore at 100% DC. Such an observation becomes significant with the increase of sonication duration (see Fig. 3). SC surfaces within the pore of untreated skin were arranged orderly and intact with parallel contours. Surfaces surrounding the pore were covered with overlapping corneocytes (Fig. 3a). After 7-minute sonication, uneven surface emerged and flakes started to appear (Fig. 3b). Although the clearing of keratinocyte is slight, the flakes are still firmly attached to the skin, which may be due to the stress induced by the bubble cavitation in the acoustic field. The disruption of the corneocytes and keratinocytes was more profound for the 15-minute and 30-minute sonication (Figs. 4c and 4d). The corneocytes were disintegrated with flakes of detached keratinocytes spreading across the surface. In addition, the pore sizes were almost unchanged (~100 μm), and neither skin burns nor large mechanical holes was found in SEM.

IV. Discussion

Ultrasound-induced convection was considered no contribution to drug transport because no difference of mannitol for transdermal penetration with and without sonication (Mitragotri et al. 2000). So usually the drug was spattered on skin for passive penetration after sonication. However, the difference becomes significant for larger molecule, such as insulin (Boucaud et al. 2002), which is due to the dominant acoustic radiation force for large product of wavelength and particle radius (King 1934). Utilizing it, the activation of nanoparticles to cancer cells can also be enhanced (Crowder et al. 2005).

Bubble cavitation is one of the major mechanisms in sonophoresis, and could also occur *in vivo* in the 5 μm sweat and sebaceous gland ducts with no histological change (Machet and Boucaud 2002). Meanwhile, the high speed jet impact at the collapse stage of cavitation bubble can also pump the liquid through a hole (Khoo et al. 2005, Lew et al. 2007). If the ratio of hole diameter to the maximum bubble size is large (as the pore in our skin sample), the jet impact occurred away from the boundary, and the bubble moves into the hole at very high speed (~24 m/s) with a considerable amount of fluid. If the distance between the cavitation site and the hole is less than the maximum bubble radius, bubble will enter the hole during its expansion phase, and a toroidal cavitation bubble will form near the edge of the hole, whose collapse may be the reason of the damage surrounding the pore entry in our SEM images. Further work is required to investigate the bubble induced by sonication, its location around the pore, and the pumping effect of particle transport, which may enhance our understanding of the role of bubble cavitation in the sonophoresis and subsequently improve the efficiency.

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High frequency ultrasound has been shown to be effective for low molecular weight drugs (< 500 Da) across human skin while the low frequency one has up to three orders of magnitude more effective at increasing skin penetration, such as interferon-γ□(17 kDa) and erythropoietin 48 kDa) (Mitragotri et al. 1995). The discrepancies of nanoparticle penetration in this study may be due to the pre-existing pores in the porcine ear skin specimens (~100 μm, much larger than the holes produced by the bubble cavitation, 1-3 μm (Machet et al. 1998), and the sweat pore, ~20 nm), long continuous sonication, and less interaction of polystyrene materials with the biological tissue. Transdermal delivery of macromolecules and vaccines is of great interest and potential with the continuous investigation.

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