Permeability Enhancement for Transdermal Delivery of Large Molecule Using Low-Frequency Sonophoresis Combined with Microneedles

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ABSTRACT: Transdermal drug delivery is limited by the high resistance of skin towards diffusion of high-molecular-weight drugs. This is mainly because of the fact that the outer layer of the skin, that is the *stratum corneum*, can prevent diffusion of molecules whose molecular weight is greater than 500 Da. Sonophoresis can be used to enhance the permeability of the skin. However, in the delivery of large molecules, ultrasound alone cannot provide sufficient permeability enhancement. In addressing this issue, we propose optimised ultrasound combined with microneedles to further increase the permeation rates. In this paper, we use porcine ear skin to simulate human skin and treat the skin samples with both ultrasound and microneedles. Further, bovine serum albumin (BSA) is used as a model of larger molecular weight molecule. Our results show that the permeability of BSA is increased to 1 μm/s with the combination of 1.5 mm microneedles patch and 15-W ultrasound output which is about 10 times higher than the permeability obtained in passive diffusion. Diffusion with only microneedles or ultrasound pre-treatment is also tested. The maximum permeability from microneedles and ultrasound treatment reached 0.43 and 0.4 μm/s, respectively. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3614–3622, 2013

Keywords: transdermal drug delivery; sonophoresis; microneedles; Franz diffusion cell; permeability; high molecular weight drug; HPLC; skin; ultrasound; diffusion

INTRODUCTION

Until the 1940s, transdermal drug delivery (TDD) method was considered as one of the most essential methods for drug delivery through the parenteral drug delivery routes.[1] At that time, the dosage forms of TDD only had topical creams and ointments, and they relied on passive diffusion of the drug molecules through skin. The physicochemical properties of the drugs were generally hydrophobic in nature because of the lipophilicity of skin[2] and because the hydrophilic drug formulations seemed to have more tendencies to vaporise than to permeate through the skin. The TDD methods can avoid the gastrointestinal and liver metabolisms which may be severe for the delivery of some proteins and vaccines. As the scope of TDD has increased over the decades, it can be considered as a main alternative to other parental drug delivery routes, for example oral delivery route.[3] With the continued development of pharmaceutical and related sciences, more drugs are being invented which may need to be delivered transdermally. The molecular weight of these drugs can vary from less than 100 Da to over 150 kDa. Several technologies have been developed to increase the permeability of these drugs in skin. But, in the delivery of large molecules such as insulin, bovine serum albumin (BSA) or tetanus toxoid, the delivery rates of the molecule are still found to be either low or undetectable according to many literatures.[4–7] Because of the desired pain-free nature of the TDD technologies,[8] the permeability increment of large molecules that just relied on one delivery method, such as passive diffusion, can be difficult. As such, researches on combining individual technology are necessary so as to multiply their advantages in enhancing the drug permeability.

In addressing these issues, we explore a way to combine ultrasound-generated cavitation with microneedles patch in the current work for delivering large molecules, both of which are chosen from well-accepted TDD methods. As well known, the TDD methods can be divided into three categories in terms of their mechanisms, namely (1) diffusion of small ions or charged molecules under an electrical field known as iontophoresis and electroporation[9,10]; (2) increase of drug solubility in the donor solution or cooperate with chemical enhancers to increase permeability[11,12]; (3) penetrate or rub the skin surface to change the structure of the skin including sonophoresis and microneedles.[13,14] Both ultrasound and microneedles create pores/holes of different scales in skin. The holes created by microneedles are generally visible through naked eyes and do not close up immediately. On the contrary, the ultrasound is focused on fluctuating the size of naturally occurring skin pores by creating cavitation in the skin. At the moment, there are very few reports on sonophoresis-enhanced delivery of large molecules because the pores created by ultrasound are often not sufficient to deliver those molecules.

The performance of the microneedles patch is also limited by its own properties, for example the length of the needles, the needle density in the patch, the geometry of each needle and materials of needles.[15,16] The microneedles not only create holes on the surface of the skin but also change the skin property (e.g., effective viscoelasticity and transport properties) of the affected area.[13,17] It will leave a good basis after the treatment so that the ultrasound-generated cavitation can become more efficient on that field. The ultrasound and microneedles can be then

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combined as an effective group. However, the other two technologies, namely chemical enhancers and electrical fields, do not seem to be readily suitable for the delivery of large molecules because the chemical enhancers are most efficient in the transportation of small molecules and the electrical fields can only deliver ionic compounds.[18]

The focus of this paper is to carry out pre-treatment of skin by microneedles so as to alter the skin property, and then apply ultrasound field on the pre-treated area to let the cavitation further increase the permeability of the skin. As a model molecule for the permeation measurement, BSA is chosen in this study. It is a common protein derived from cows and has a molecular weight of over 60,000 Da. The reason of choosing BSA for the experiments is because BSA is a hydrophilic molecule and its molecular size ranges between the sizes of small peptide molecules and large vaccines. Also, it does not metabolise in skin. Because of its molecular cut-off, BSA should not diffuse passively through the *stratum corneum* (*SC*) layer. However, if the skin is treated with other factors such as microneedle, the molecule may pass through. To help BSA passing through the *SC* layer of the skin, the sonophoresis (or phonophoresis) combined with microneedles patch method is employed as discussed further.

As well known, sonophoresis uses ultrasound to enhance the permeability of the skin. The first study in this area was carried out for the treatment of polyarthritis (a type of arthritis involving a minimum of five joints simultaneously) using hydrocortisone ointment combined with ultrasound in 1950s after which this method has been widely used in the treatment of many other joint diseases and bursitis.[19] The two main adjustable parameters of ultrasound are the intensity "*I*" (power on unit area) and frequency "*f*" (number of cycles per second).[20] These two parameters work in a synergetic manner. As Tezel et al.[21] reported, there is threshold intensity for every different frequency. For example, the threshold intensity is 0.11 W/cm^2 at 19.6 kHz and greater than 2 W/cm^2 at 93.4 kHz. Once the intensity exceeds its threshold, the enhancement of drug delivery increases significantly as well. Although the experiment of Tezel et al.[21] was based on the measurement of porcine skin conductivity, it gives an idea that in the delivery of a specific drug, ultrasound output parameters can be optimised by careful selection of an intensity range for a specific frequency. For example, at a frequency of 1 MHz, the permeability increase for delivery of mannitol (182 Da) through rat's skin is not detectable at a frequency of 0.1 W/cm^2 in comparison to passive diffusion of the drug. However, it increases by twofold at an intensity of 1.5 W/cm^2 and fourfold at an intensity of 2 W/cm^2.[22,23] Besides the intensity and frequency of ultrasound, the scale of the permeability enhancement for a specific molecule is also strongly connected to its molecular size. For cortisol (382 Da) under the same conditions (1 Mhz, 1.5 W/cm^2), the increment of permeability is less than 20%.[22] Besides, the duty cycles, treatment time and the distance between ultrasound transducer and target also need to be considered carefully for specific ultrasonic application.

The primary mechanism in sonophoresis application is a cavitation effect, which have been discussed in length by many authors.[14,24] When the ultrasound waves compress and tense a liquid, the liquid pressure falls below its vapour pressure which forms the cavitation. The cavitation is divided into two types, namely stable and transient cavitations, which are discriminated by how long the bubbles survive.[25] The cavitation generated during high-frequency ultrasound treatment is much smaller in size compared with those for low-frequency condition because of the relationship between the frequency and the bubble radius, that is $C = f \times r$, where C is a constant determined by the properties of the solution, f is the frequency of the ultrasound and r is the radius of the bubble.[26] For example, bubbles generated in water caused by 20 kHz ultrasound is typically 150 μm in radius, but it is only 1 μm when the ultrasound frequency is increased up to 3 MHz.[27] The larger bubbles can cause more disruption when they burst. For this purpose, 20 kHz is chosen in most sonophoresis TDD experiments.[24]

There are other factors that may affect the skin permeability. The most obvious phenomenon during the ultrasound application is the temperature rise of the skin. The skin absorbs the mechanical energy of sound field. This increases the temperature which may be significant when the ultrasound frequency and intensity go up. This means that the energy would be stored in skin rather than transmit through[28,29] affecting the skin permeability. The parameters of ultrasound inputs must be kept to a safe range because high temperature can cause skin injury. It has been reported that when the temperature reaches 43°C or higher and stays at that level for 60 min or longer, it can restrain cellular reproduction. If the temperature increases to 56°C, it can cause irreversible cell death and necrosis.[30] This is the main reason why we choose to apply ultrasound prior to the diffusion experiment rather than applying it simultaneously.

In this paper, we use low-frequency sonophoresis, which indicates the ultrasound frequency between 20 and 100 kHz. Unlike high-frequency sonophoresis, the low-frequency sonophoresis research has only been introduced over the last 10 years or so.[25] Researches have shown that low-frequency ultrasound have much better effect on drug delivery enhancement (both low- and high-molecule-weight drugs) than high-frequency ultrasound (beyond 1 MHz) which has also been proved by the cavitation theory mentioned earlier.[14] Large proteins start being used as target drugs: Boucaud et al.[31] report that the blood level of *in vivo* rat drops to half when one applies insulin (5.8 kDa) with ultrasound (2.5 W/cm^2, 20 kHz, 15 min).

Microneedles patch is a kind of technology which sits at the interface between transdermal patches and hypodermic needles, attempting to gain the advantages and eliminate the disadvantages of each.[32,33] The idea of microneedles comes from the patent of Gerstel and Place[34] which gave the concept to make micropores in the skin. However, the first study of using microneedles to enhance TDD process was conducted in 1998.[35] Following this work, microneedles technology has developed rapidly and extended greatly for pharmaceutical applications such as TDD. Compared with hypodermic needles, microneedles are pain free or significantly reduce the pain depending on the length of the needles. Gill et al.[36] have used different lengths of microneedles from 480 to 1450 μm, tested them on human volunteers and found that the needle lengths below 750 μm is painless and bloodless. There are generally two types of microneedles, namely the solid microneedles and the hollow microneedles. There are many reports on the delivery of large molecules using microneedles patch. Martanto et al.[37] reported the delivery of insulin through rat's skin using 1000 μm length microneedles patch. They used the microneedles patch to repeatedly pierce the same site, and as a result, the insulin blood level dropped to one quarter. Dissolving microneedles have also been used for delivering large molecules such as lysozyme (14 kDa) or BSA (60 kDa), but the drug loading on the microneedles

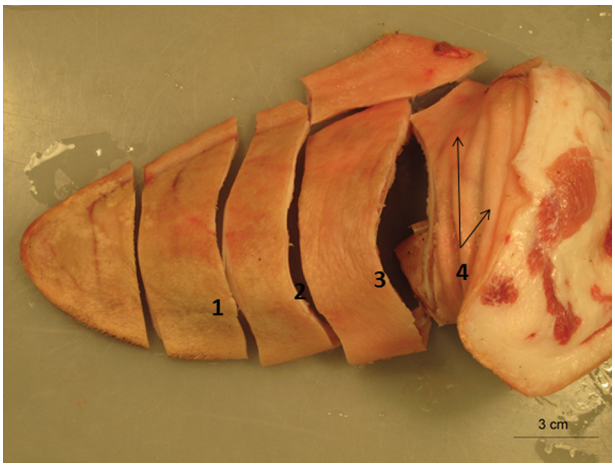

Figure 1. The porcine ear is sliced into four sections. The thickness of these sections increases from section 1–4.

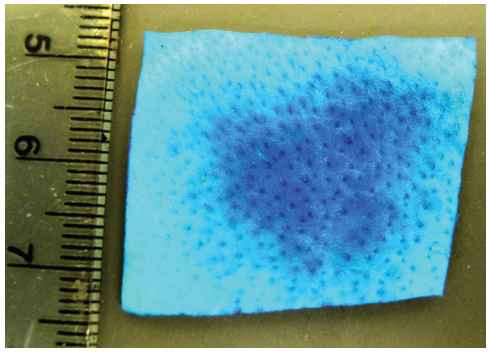

Figure 2. The identification for the ultrasound field. The deep blue area shows the ultrasound-generated cavitation-affected area.

patch is typically less than 1–2 mg which seem to limit their application.[38]

In our study, we use solid microneedles because it is more rigid in structure in comparison to the hollow microneedles. The microneedles can create a porous basis as well as dents and other structural changes on the skin for diffusion rate to increase. It weakens the resistant functionality of the *SC* layer and exposes parts of the underneath epidermis to the molecule of interest. The ultrasound treatment is then applied on the microneedles-pre-treated area to further enhance the skin permeability. The output of ultrasound uses continuous wave mode to generate more bubbles in the limited time duration. These bubbles will be in contact with both *SC* layer and microneedles-pre-treated area so the permeability is significantly increased. The whole permeation study is based on a Franz diffusion cells system so the amount of the BSA passes through the skin can be quantified. Further, only low-frequency ultrasound is used in this study.

MATERIALS AND METHODS

Materials

Bovine serum albumin and methylene blue were obtained from Sigma–Aldrich (Gillingham, Dorset, UK). Trifluoroacetic acid (TFA) and acetonitrile were bought from Fisher Scientific (Loughborough, UK) for using them as the high-performance liquid chromatography (HPLC) mobile phases. All deionised water for use in permeation experiment was purified using Milli-Q System (Billerica, Massachusetts). Porcine ear skins were purchased from a local abattoir. The porcine ears were collected from 5–6-months-old piglets. The skin samples were then kept in a cool-box for the transport from the abattoir to our laboratory. The ears were cut into four sections as shown in Figure 1. Section 1 is too thin and it seems to get damaged during the process of separating the skin from the cartilage in the ears. Therefore, section 1 was not used in our experiments. Section 4 was also rejected because of the existence of excess underneath fats and muscles. These tissues are likely to affect the thickness of the skin samples varying in a wide range and will probably cause inaccuracies during the permeation results.

Sections 2 and 3 were cut into small pieces and wrapped in foil papers and then flash frozen in a tank filled with liquid nitrogen. According to the skin graft preservation procedure,[39] the skin samples were dipped in the liquid nitrogen until it stopped boiling (about 40 s) which indicates that the temperature of the skin has reached –196°C. The skin samples are then kept into a container in the freezer that is set to –22°C. Before the permeation experiments, these samples are allowed to stay in room temperature for 2 h until which they are fully thawed. A surgical scalpel is used to separate full thickness porcine ear skin from the underneath cartilage. To assure the integrity of the skin samples, some of the connective tissues under the skin are removed carefully from the cartilage to avoid irrelevant tissues.

Treatment on Skin with Ultrasound and Microneedles

A commercially assembled ultrasound system (Branson Digital Sonifier 450 Danbury, Connecticut), which includes an ultrasound generator and an ultrasound transducer, is used to treat the skin in this work. The frequency of the ultrasound is fixed at 20 kHz, whereas the input powers are varied between 4 and 400 W. To ensure that the sound field indeed affects the localised transport regions (LTRs),[5] the transducer is set to 12 W and kept approximately 1 mm away from the skin surface. The ultrasound is then applied to the skin for 10 min and its effect is visualised as follows. The skin sample is completely merged in 100 mL water and adhered to the bottom of the beaker. One drop of methylene blue is then dissolved in the water which is distributed immediately by the ultrasound transducer. Because of the LTRs effect, the sound field will not spread on the skin consistently. However, as the skin is merged in the methylene blue solution, the entire skin surface turns into blue. But, the area that is most affected by the sound field shows deeper blue than the surrounding untreated area which indicates that more dye molecules has permeated into the regions through the skin area that are affected most by the sound field. This is shown in Figure 2. In terms of choosing the most affected regions for permeation study, the centre of the skin sample is employed.

The microneedles patches were purchased from nanoBio-Sciences (Sunnyvale, California). The length of the microneedles is 1500 and 1200 μm with 31 and 43 individual microneedles on each patch, respectively. The 1500 μm microneedles patch is shown in Figure 3.

To identify the hole size created by the microneedle, a staining experiment is conducted which allows visualisation of the

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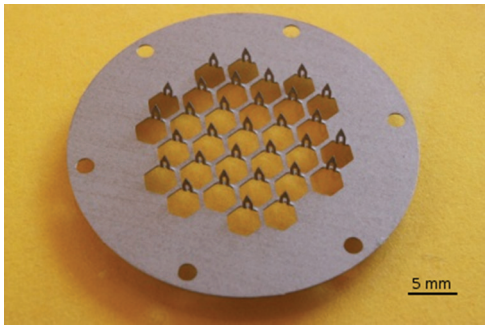

Figure 3. The 1500-μm long microneedles from Adminpatch.

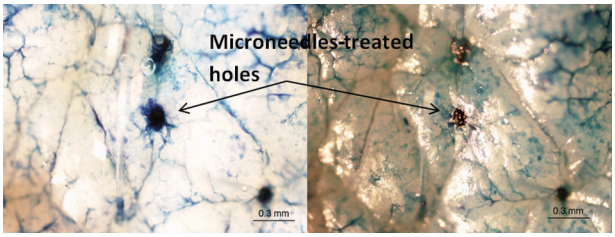

Figure 4. Microneedles-treated holes in porcine skin where the left picture shows the holes immediately after staining, whereas the right picture shows the size of the same hole after 2 h.

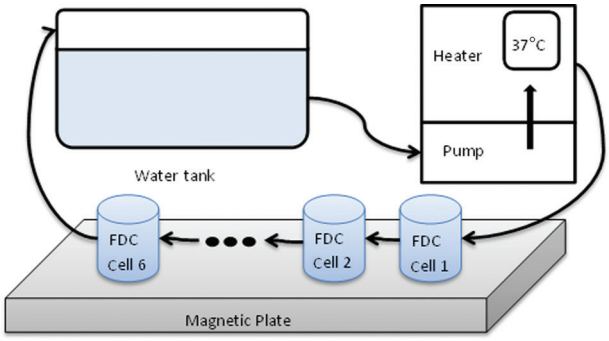

Figure 5. The full set-up of the Franz diffusion cells system. The four components of the system are marked in the picture.

holes and confirm that the microneedle has pierced the skin. For this purpose, the methylene blue solution is diluted to 10% (v/v). The skin sample is treated with the microneedles patch for 5 min, and then merged in the dye solution for 1 min. The dye solution is then washed off with deionised water and the skin sample is observed under the microscope. Figure 4 shows some typical holes size which becomes smaller after 2 h.

Diffusion Cells

A Franz diffusion cell, which is commonly used for measuring drug permeability in skin, was used in this work to determine the permeability. It is consisted of four parts: the water tank, the heater (VTC 200; Logan Instrument Corporation, New Jersey), the magnetic plate (FDC-6; Logan Instrument Corporation) and the diffusion cells (Fig. 5). The water in the water tank is pumped through the whole system. To simulate body temperature, the water is heated to 37°C which warms all the diffusion cells by going through their jacketed compartments. The real temperature may be different by a margin of ±1°C from the set value because of the inaccuracies in the sensor.

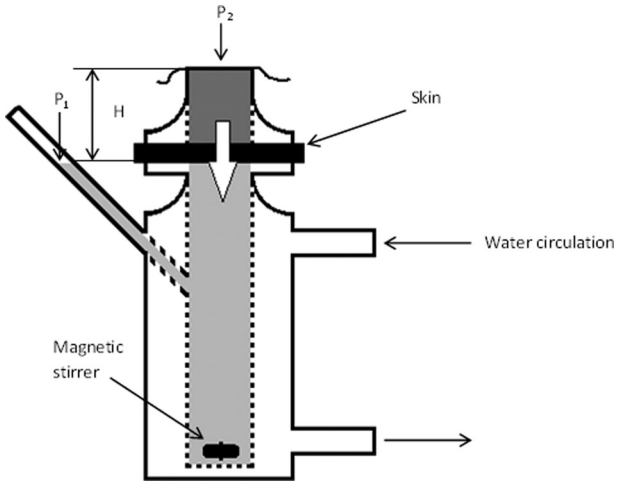

Figure 6. The individual FDC cell where the donor compartment is at the top, the receiving compartment is at the bottom and the skin sample is in between them. A water circulation is used to keep the receiving compartment at 37°C.

For the individual cells (Fig. 6), there are two compartments which are the main parts of the cell: the donor compartment on the top and the receiving compartment at the bottom. The skin sample sits in between of the two compartments and it is fixed using a clipper. A magnetic stirrer is used to mix the receiving solution which represents the blood circulation beneath the skin.

For conducting the permeability measurements, the donor compartment is filled with BSA solution of a certain concentration while the receiving compartment is filled with deionised water. The liquid surface level is different between donor compartment and the receiving compartment so that a pressure head exists. This pressure head can generate convection effect which may affect the permeability. To balance the pressure difference, a Parafilm is used to seal the donor compartment to keep pressure at $P_2 = P_1 - \rho g H$, where P_1 is equal to standard atmosphere, P_2 is the pressure under the Parafilm, ρ is the density of the BSA solution, g is acceleration because of gravity and H is the pressure head. The HPLC is used to analyse the BSA concentration of the sample taken from the receiving compartment. The samples are mixed with 95% of mobile phase A (0.1%, v/v, TFA in H_2O) and 5% mobile phase B (0.08%, v/v, TFA in acetonitrile) and pumped through the HPLC column at a rate of 10 μL/min. A calibration curve is made beforehand to identify the relationship between the light absorbance and the concentration. It also indicates the retention time of BSA curve which is at 13 min in the run. To calculate the permeability of the skin, the Fick's law is used:

$$J_{SS} = \frac{Q}{AT} = \frac{D\Delta C_v}{h} \quad (1)$$

where J_{ss} is the total flux, Q is the total mass of BSA in the receiving compartment, A is the affected diffusion area which is fixed to 1.33 cm^2, T is the time interval between each sample, ΔC_v is the concentration difference between the donor and the receiving compartment and D is the diffusion coefficient of the skin. The volume of the receiving compartment is 5 mL in this case. Using the concentration data acquired from HPLC,

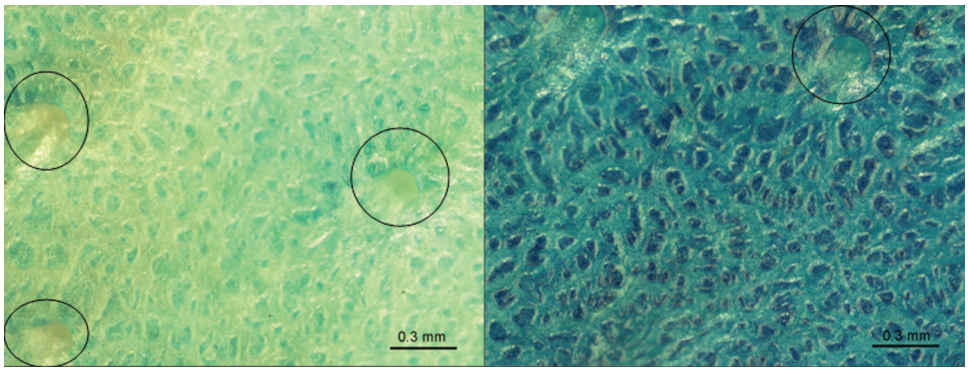

Figure 7. The pictures show the difference on skin surface before and after 10 min of ultrasound treatment. The left picture shows the skin sample before treatment. Hair follicles are marked with black circles.

the total amount of BSA in the receiving compartment Q can be calculated. After calculating the total flux, the permeability of the skin k_p is deduced from the following equation:

$$k_P = \frac{J_{SS}}{\Delta C_V} \quad (2)$$

where the ΔC_v is calculated just using the concentration in the donor compartment because the concentration of BSA in the receiving compartment is negligible compared with the concentration in donor compartment.

All the diffusion experiment and temperature measurement data are presented as arithmetic mean values ± standard deviation (SD). For each set of experiment (passive, microneedles and ultrasound), six individual skin samples are used to calculate the mean values. The statistical data are presented directly in the figures.

RESULTS AND DISCUSSIONS

Staining Experiment

As mentioned before, a series of staining experiments has been performed on typical skin samples to confirm that the microneedles create holes in the skin under normal thumb pressure. The porcine skins that were bought from the local abattoir were dehaired by the butcher. The skin samples seem to provide a clear vision of the hair follicles besides the pores on the skin surface as shown in Figure 7. Any micro-pores created by the ultrasound cavitation are undetectable. But a dye solution can reveal the ultrasound effect on the treated area on the skin surface. Furthermore, by magnifying the ultrasound-affected area, a qualitative comparison of skin surface before and after the ultrasound treatment can be performed under the microscope. For this purpose, two samples are obtained from the same piece of skin. They are immersed in the dye solution with and without ultrasound treatment, respectively. In the former case, the output power of ultrasound is set at 12 W, whereas the distance between the ultrasound horn and the skin surface is set at 1 mm. The skin sample with ultrasound treatment shows higher permeability. The results of the diffusion difference are compared in Figure 7.

Measurement of Passive Diffusion of BSA

At this stage of the experiments, the passive diffusion for BSA is tested for different concentration of the molecule. In principle, the skin should block any molecules that are larger than 500 Da[40] with a partition coefficient between 1 and 5[41,42] passing through its top layer. BSA has molecular weight of 60,000 Da which is much higher than 500 Da, and as such, the BSA permeability should be approximately zero in the passive diffusion experiment. However, because of the fact that there are

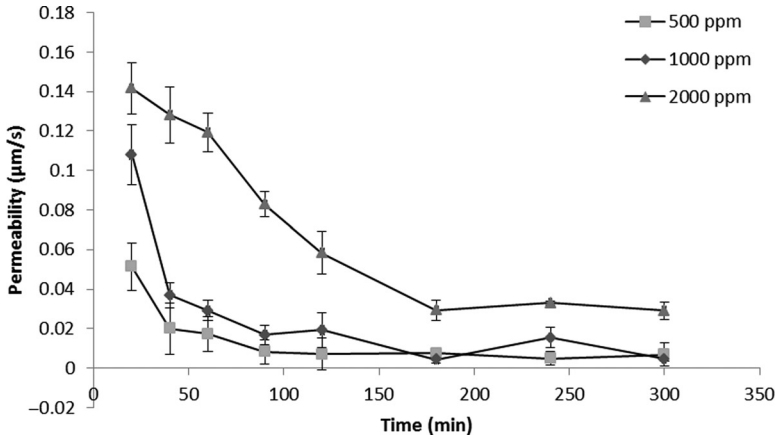

Figure 8. Passive diffusion with different BSA concentrations of 500, 1000 and 2000 ppm (results represent mean ± SD values based on data from six skin samples).

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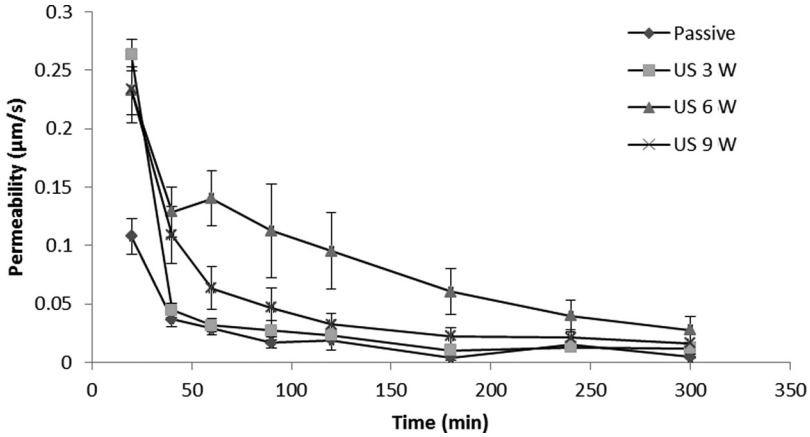

Figure 9. Bovine serum albumin permeability because of combined passive diffusion and ultrasound power at 3, 6 and 9 W for 5 min for BSA concentration of 1000 ppm (results represent arithmetic mean ± SD values based on data from six skin samples).

many imperfections in the skin and the skins are treated by the butcher, some of the BSA molecules still diffuse through the skin. In the skins that were used in these experiments, an average permeability value of approximately 0.11 µm/s has been obtained initially. A BSA solution of 1000 ppm is used as a standard concentration in all permeation experiment. But to discriminate the concentration effect in the permeation study, passive diffusion experiments that involve three different concentrations of BSA solution (500, 1000 and 2000 ppm) are conducted at the beginning. In each permeation study for a certain concentration, six skin samples are used and the results are shown in Figure 8. Although the donor concentrations vary significantly, the results show that the permeation values are not affected considerably by the large variation of BSA concentrations which moves from approximately 0.06 µm/s for 500 ppm to approximately 0.14 µm/s for 2000 ppm.

Diffusion with Sonophoresis

Sonophoresis can greatly increase permeability for small molecules which has been reported in a number of papers.[23,43] At 20 kHz, the bubbles generated by ultrasound have their maximum size[26] so they can produce the highest damage to the *SC* layer. But, in the delivery of large molecule such as BSA, a relatively high output power is essential to create enough cavitation. At lower power range, the permeability is not significantly increased. As Bangtao et al.[44] have reported that at 20 kHz 0.5 W output power, the BSA permeability is below 0.1 µm/s for 8 h duration. In Figure 9, the results of passive diffusion and ultrasound pre-treatment at different output powers (3, 6 and 9W) have been shown for a 5-h experimental duration. In each permeation study, six skin samples are involved, and they have been treated with ultrasound at certain power for 5 min. The results indicate that at low output power range, the ultrasound-enhanced permeability is not remarkable. At 9 W output power, the permeability reaches about 0.26 µm/s, whereas non-ultrasound-treated permeability is about 0.11 µm/s. The permeability is doubled but the increment is still not significant.

To optimise the sonophoresis effect, the power of ultrasound must be increased to a higher level and a longer pre-treatment time. In other words, the ultrasound output must reach the threshold which has been mentioned earlier. To find this threshold, a permeation study on the different output powers is conducted. In Figure 10, the ultrasound output power is varied

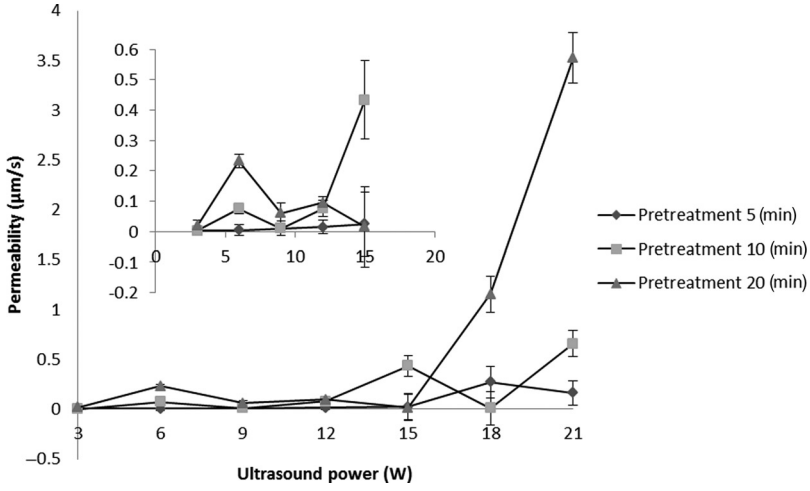

Figure 10. Sonophoresis effect with different power output from 3 to 21 W and pre-treatment time at 5, 10 and 20 min (results represent arithmetic mean ± SD values based on data from six skin samples).

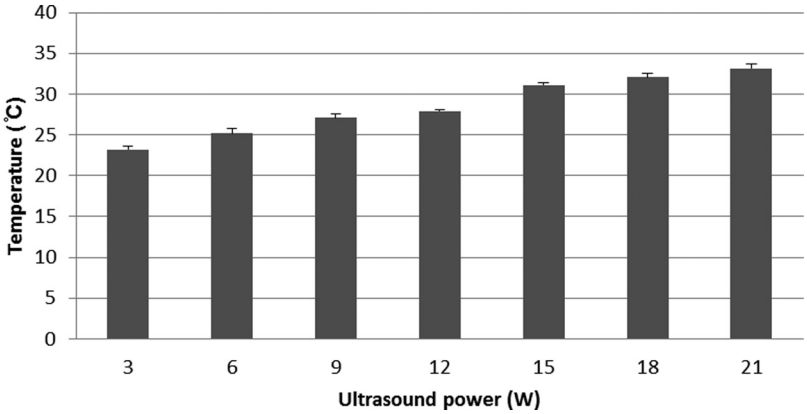

Figure 11. The temperature changes with different ultrasound power for 10 min. The reference room temperature is 20°C (results represent arithmetic mean ± SD values based on data from six skin samples).

between 3 and 21 W. Further, the pre-treatment time is varied between 5 and 20 min. As evident in the figure, at 21 W with 20 min pre-treatment time, the permeability is significantly increased to approximately 3.5 μm/s. However, the skin sample is damaged at a visible level, which means that high temperature and pain will be caused as well if these are applied to a human. To avoid the experiment results go into the decoupling stage where the skin is irreversibly damaged, the power must be chosen so as to maintain the ultrasound treatment at a safe level.

In Figure 11, the temperature accompanied with ultrasound pre-treatment is reported. At 10 min treatment time, no significant temperature increase on the skin surface has been recorded. The ultrasound effect is not high enough even in a relatively high power output. At 21 W output, the temperature reaches approximately 33°C which is below the human body temperature. In that case, 10 min treatment with ultrasound can be regarded as safe.

Another issue which needs to be pointed out about the temperature rise is the trend of the temperature curve. If the temperature suddenly rises to a certain level, it may cause uncomfortable experiences to humans. In Figure 12, temperature change under 15 W ultrasound treatment for 10 min is recorded every 10 s. The results show that the temperature rises smoothly at a constant rate of temperature increase. In the same figure, the temperature decrease of the skin sample is recorded. As evident, compared with the cooling curve, the temperature increment rate is quite slow. In such case, the experiences under the ultrasound treatment should be mild.

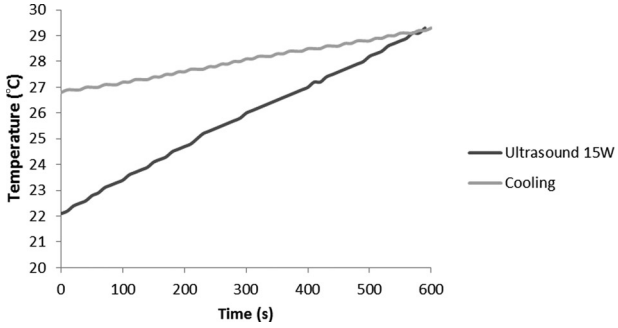

Figure 12. Temperature change during and after 15 W ultrasound treatment for 10 min.

Diffusion with Microneedles Patch

To further increase the permeability, a microneedle patch is used. Before combining the ultrasound with microneedles patch, a permeation study which is solely based on the microneedles is conducted. Devin et al.[45] reported that the permeability of BSA using a 150-μm long microneedle patch is about 0.02 μm/s. As the permeability increase is nearly undetectable for BSA with smaller microneedle (as found in our experiments but not published anywhere), a longer microneedles patch is preferred in this study. For the results in Figure 13, the microneedles patches with 1.5 and 1.2 mm lengths are applied to the skin sample for 10 min under a certain pressure of 1 MPa. A 5-h permeation study is then conducted similar to the passive diffusion experiments.

In comparison with Figure 10, the microneedles patch increases the permeability to about 0.43 μm/s, whereas the ultrasound at 15 W can increase the permeability to about 0.4 μm/s. From Figure 10, we find that the best ultrasound output power should be 15 W with 10 min pre-treatment time. Although the 21 W can give higher permeability according to its performance at 20 min application time, it reaches the decoupling level at this condition and should be avoided in practice. The 15-W output power with 10 min pre-treatment time turns out to be the best parameter for enhancing the permeability and it is also at a safe level. These ultrasound output parameters are then applied on the microneedles-pre-treated skin to investigate any further permeability enhancement.

Ultrasound Combined with Microneedles Patch

In Figure 14, different ultrasound powers with a 10-min treatment time are applied on the microneedles-pre-treated basis. The results in Figure 14 show that the permeability is significantly increased when using the 15-W ultrasound output power and 10 min treatment time combined with the microneedles. It also indicates that the thresholds of the synergetic power at 20 kHz are in between of 12 and 18 W. Therefore, in cooperation with microneedles pre-treatment, the permeability reaches a much higher level, which is approximately 1 μm/s.

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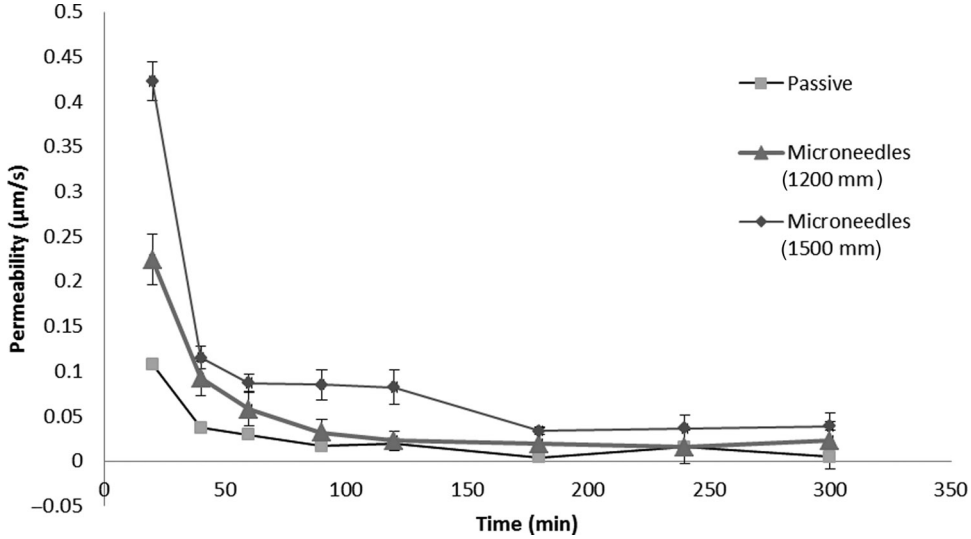

Figure 13. Microneedles pre-treatment for 10 min (results represent arithmetic mean ± SD values based on data from six skin samples).

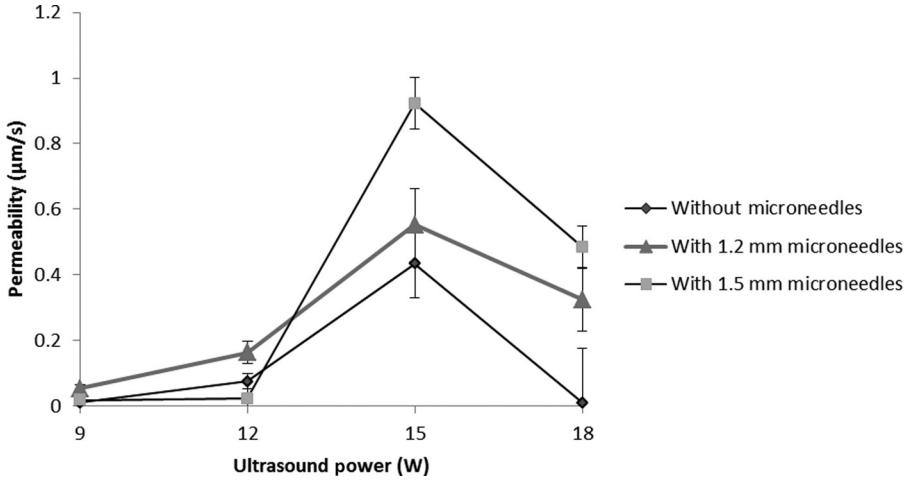

Figure 14. Different ultrasound output power with 10 min treatment time combined with 1.2 and 1.5 mm microneedles patch (results represent arithmetic mean ± SD values based on data from six skin samples).

CONCLUSIONS

The idea of combining sonophoresis with microneedles patch provides a feasible way for the delivery of large molecules. The permeability of BSA, which has a relatively large molecular weight, is proved to be significantly increased as shown in this paper. For the purpose of this paper, the skin samples are pre-treated with both ultrasound and microneedles patch for 10 min each. The BSA permeability reaches 1 µm/s which is a reasonable amount for delivering small dosage of the molecule. It also indicates the possibility of transporting large molecules through human skin in future. Molecule such as insulin which is much smaller than BSA should have larger permeability if similar approach is used. The combination of microneedles patch and ultrasound may become a painless alternative to the hypodermal injections for delivering large molecules.

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