Potential of combined ultrasound and microneedles for enhanced transdermal drug permeation: A review

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Abstract

Transdermal drug delivery (TDD) is limited by the outer layer of the skin, i.e., the *stratum corneum*. Research on TDD has become very active in recent years and various technologies have been developed to overcome the resistance of the *stratum corneum* to molecular diffusion. In particular, researchers have started to consider the possibility of combining the TDD technologies in order to have further increase in drug permeability. Microneedles (MNs) and ultrasound are both promising technologies. They achieve enhancement in drug permeation via different mechanisms and therefore give a good potential for combining with each other. This review will focus on discussing the potential of this combinational technique along with other important issues, e.g., the mechanisms of ultrasound and MNs as it is these mechanisms which are coupled via the two systems (i.e. MNs and ultrasound). We discuss the possible ways to achieve this combination as well as how this combination would increase the permeability. Some of the undeveloped (weaker) research areas of MNs and sonophoresis are also discussed in order to understand the true potential of combining the two technologies when they are developed further in the future. We propose several hypothetical combinations based on the possible mechanisms involved in MNs and ultrasound. Furthermore, we carry out a cluster analysis by which we determine the significance of this

combinational method in comparison with some other selected combinational methods for TDD (e.g., MNs and iontophoresis). Using a time series analysis tool (ARIMA model), the current trend and the future development of combined MNs and ultrasound are also analysed. Overall, the review in this paper indicates that combining MNs and ultrasound is a promising TDD method for the future.

Keywords: Transdermal drug delivery, sonophoresis, microneedles, iontophoresis, chemical enhancers, permeability, autoregressive integrated moving average, cluster analysis

1. Introduction

Transdermal drug delivery (TDD) methods intend to deliver drug molecules to the blood circulation at a controlled rate for which the molecules need to pass through different sub-layers of the skin. TDD is developing fast and there are now many approved drugs for TDD, e.g., nineteen (19) drugs have been approved by the Food and Drug Administration, USA [1]. The potential of TDD for treating human diseases is also huge. For example, TDD can provide prolonged treatment time in the cure of chronic diseases while maintaining the permeation of the active drug molecules at a controlled level [2]. The diseases may be either psychological or physiological, and may need TDD ranging from nicotine patch for smoking cessation to the treatment of eczema [3, 4]. However, the full potential of TDD is not fully exploited yet, which is evidenced by the fact that new questions continue to be asked on how to develop the TDD methods further, for example, to resolve specific issues and/or incorporate the latest technological advances. For instance, it has been asked if it is possible to make functionalised delivery system for vaccines that can be applied in a simple way such as topical administration [5]. To develop a TDD method for clinical purposes, one may require a significant amount of finances and many technical impediments would need to be resolved [6]. For example, it is evident that the market of TDD products has developed very

fast and they were worth a market value of US $21.5 billion in 2010 which accounts for more than 12% of global drug delivery market. The development of the TDD market is predicted to reach US $31.5 billion by 2015 in which US $3 billion belongs to transdermal patch market [7]. However, the diversity of the drugs that could be delivered and various applications of these TDD techniques for treating human diseases are still limited.

Despite the commercial successes of the TDD methods, further development and success of these methods cannot solely depend on the transdermal patches. Improvement on the drug delivery efficiency and increment on the numbers of applicable drug molecules need to be achieved in the future by extending the TDD technology in multiple ways. In these regards, one of the main technical obstacles that should be overcome is the low efficiency on delivering large molecules such as proteins, vaccines, and micro-particles [8] using the TDD methods. Microneedles (MNs) [9] and ultrasound [10] are two TDD techniques which work using different principles/mechanisms but they have shown great potential to remove this obstacle either on their own or in combination with each other. There are a number of publications now which have reviewed these two technologies on their own [11, 12, 13, 14, 15]. There are also some recent studies where MN and ultrasound have been combined to increase skin permeability of large molecule [16, 17]. However, there is a lack of systematic review which discusses thoroughly the potential of combining MNs with ultrasound for enhanced drug permeation. Therefore, this review will focus on discussing the possible ways by which these two technologies could be combined. The first section of this paper will focus on explaining why the combination of MNs and ultrasound is important for TDD. The second and third sections will review the mechanisms of ultrasound and MNs, respectively, as these are the keys in the success of a TDD method that combines MNs and ultrasound. The fourth section will discuss the possible ways of combination and try to suggest what combinations

one may be interested in the future with the help of a cluster analysis. The last section of the paper is the conclusion section of the paper. The scope of this paper is discussed further in detail later in this section.

1.1 Roles of TDD

In order to provide further context to this review paper, we discuss the roles of TDD method briefly in this section. Not until the 1940s, has the TDD been specialized as one of the most essential drug delivery methods including the parenteral delivery (hypodermic injections) and oral formulations (solutions, suspensions, tablets and capsules) [18]. The main advantages of TDD over drug delivery through other routes are that: (a) TDD is user friendly, so that it can prevent needle phobia and avoid the pain perceived during the parenteral delivery [19], (b) TDD can dodge the gastrointestinal and liver metabolisms which are the most common issues in oral drug delivery [20] and, (c) TDD can provide long-term treatment without causing significant inconvenience, e.g., patients do not need to carry bulky medical instruments during the intravenous therapy which usually takes many hours [21]. In the past, the TDD methods mostly involved the uses of skin ointments and creams until a great progress was made in the 1980s when a transdermal patch was first introduced for the treatment of space motion sickness aimed at delivering scopolamine by attaching the transdermal patch on the back of the ear [22]. In general, the transdermal patches can prevent evaporations during treatment as well as achieve control rates of drug delivery [23]. However, their mechanism for drug delivery is based on passive diffusion. For this reason, the outermost layer of the skin, i.e., the *stratum corneum* (SC), restricts the choice of the drug molecule that can be administrated. For example, the molecular weight (MW) cut off for these molecules is generally taken to be under 500 Da [24] while their partition coefficient K_{ow} should be between 1 to 5 [25, 26].

1.2 Different TDD technologies

It is a matter of fact that the transdermal patch is a low efficient method in terms of drug permeability and area of skin covered by drug transport. However, there are a number of other technologies which particularly aim to increase drug transport rate and they may extend the diversity of the drug molecules that may be used in TDD (e.g., microneedles [27], sonophoresis [28] and iontophoresis [29]). All of these technologies are non-invasive or minimally invasive and, thus they provide painless drug administrations.

The technologies that aim to enhance the permeability of the drugs through the skin as compared to transdermal patches alone can be grouped broadly according to the following five classifications: (i) methods that adjust the physicochemical properties of the drug molecules or increase the chemical potential of the drug solution to acquire better delivery rate, e.g., prodrug [30]; (ii) methods that tentatively alter the skin structure or modify the drug/skin partition coefficient to reduce the resistance of *stratum corneum*, e.g., chemical enhancers [31, 32]; (iii) methods that deliver drugs or microparticles directly into skin with the help of particle accelerator, e.g., gene guns [33, 34, 35, 36]; (iv) methods that use a gradient field (e.g., pressure gradient, electrical charge, any others) to induce convective flow increasing drug delivery rate, e.g., iontophoresis [37] and sonophoresis [38]; and, (v) methods that physically disrupt or damage the skin to create new pathways which allow the drug molecules to be delivered through the skin barrier, e.g., MNs [39]. These five approaches are shown in more detail in Fig. 1. From the figure we can see that some TDD techniques may increase the diffusion rate via multiple mechanisms (sonophoresis, electroporation, etc.) while others may work similarly under the same categories. The combination of more than two or more than two techniques under the same category may not be able to yield a

promising permeability increment due to the possible redundancy/suppression of a particular mechanism in presence of another, e.g., microneedles and SC removal methods are both under category (iv) that aiming to bypass SC layer physically, thereby combining these two methods will be unnecessary. On the contrary, some TDD approaches indicate more potential for combinational methods because there are improved possibilities for them working in a synergetic way with another approach. Because the categories in Fig. 1 are subjectively divided, they are not necessarily able to provide every possible patterns of combination.

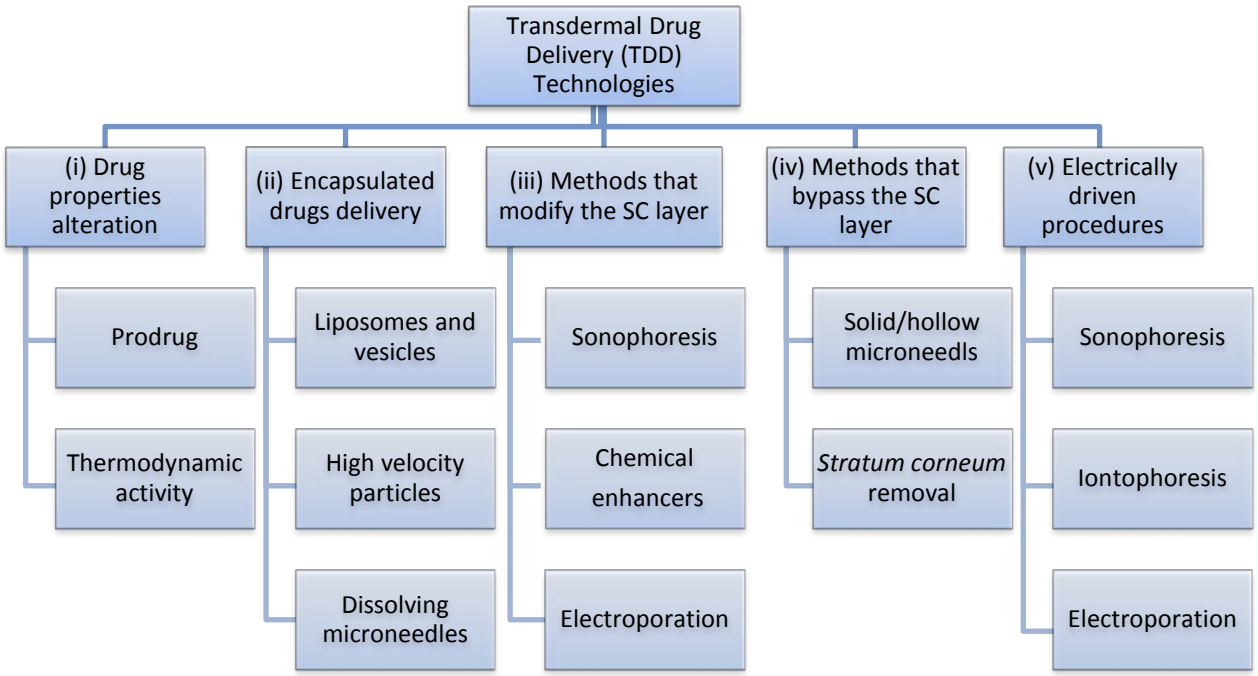

Fig. 1 Current TDD technologies can be presented in five broad branches

As stated earlier, the existing work in the literature suggests that it is possible to combine more than one method for enhancing drug permeability and there is a significant amount of work on different combinational approaches [40, 41]. However, it needs to be pointed out that the researches on the development of individual technology are very important for the development of the combinational methods because the researches on the individual method

can provide better understandings and stronger bases for the applications of these technologies. These improvements are crucial factors to ensure diversity and quality of the combinations.

The ultrasound and MNs combination has covered four branches (categories ii, iii, iv and v) in the Fig. 1 which suggests that there could be much more possible forms of combination in the future. To have better understanding on the possible combinations between ultrasound and MNs, a detailed review based on the mechanisms of both technologies are necessary which has been carried out on following sections. In order to discover more opportunities in the ultrasound and microneedles combination, the main mechanisms of these two techniques will be reviewed individually. However, there are also many other minor factors among those mechanisms which could be important in some circumstances or will become significant factors when accuracy of the TDD is taken into account. Therefore, these factors and their main mechanisms will be discussed (fourth section).

2. Ultrasound applications in TDD

The ultrasound participated applications cover many cross-cutting research areas which include physics, chemistry, biology, engineering, and others. One of the main areas where ultrasound has been employed is the medicine, as exemplified by a large number of publications between 1975 and 2013 (Fig. 2). During this period, approximately 210,540 publications have appeared which relate to the use of ultrasound in medicine alone whilst the total numbers of papers relating to all areas of ultrasound applications are approximately 283,430. The number of papers of ultrasound applications for medicine only was 941 in 1975 and it reached 13,470 in 2013 which suggests an average 7.25% increment in the number of publications each year.

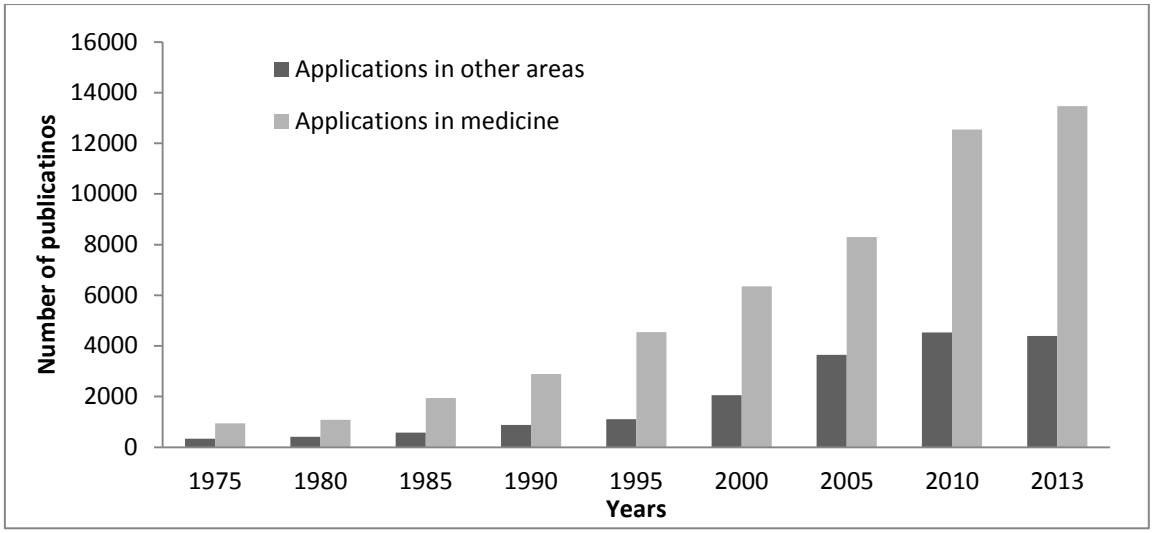

Fig. 2 The numbers of publications of ultrasound for both applications in medicine and all other areas (all results searched using Scopus [42]).

Generally, the ultrasound applications in medicine depend on the power and frequency of the ultrasound output [43]. The intensity of the ultrasound is a crucial parameter which determines its usage for either diagnostic or therapeutic purpose. The diagnostic ultrasound must have a relatively low intensity to reduce any adverse effect to human body whilst high intensity ultrasound can damage tissues via cavitation and high temperature. The ultrasound applications classified according to their frequencies and intensities shown in Table 1.

Table 1. The applications of ultrasound sorted by different parameters

Diagnostic ultrasound (real time medical imaging [44])	Physiotherapeutic ultrasound (bone healing [45])	Sonophoresis (transdermal drug delivery [46])	High intensity focused ultrasound (ultrasound blade [47])
Intensity: Low → High			
1-18MHz	1-3MHz	20kHz-3.5MHz	1-5MHz
Non-cavitation		Cavitation involved	
Thermal effect: Low → High			

According to Table 1, sonophoresis (or phonophoresis) can be defined as an ultrasound application which has sufficient intensity to reduce the resistance of skin but keeps the temperature within a safe range. The first reported application of sonophoresis was used to treat polyarthritis by using hydrocortisone ointment combined with ultrasound in 1950 [48]. Since then this method has been widely used in the treatment of many other diseases including bone joint diseases and bursitis [49]. Although this approach is recognized by scientists, a number of issues (e.g., how to choose the parameters of ultrasound) continue to pose problems in sonophoresis.

Besides the intensity and frequency of ultrasound, there are other parameters such as the duty cycles of the ultrasound, the treatment time and, the distance between ultrasound transducer and target, which also need to be considered for specific ultrasonic application [50]. Although they are often treated as minor factors [51], their importance cannot be underestimated and could become more useful in the future.

2.1 Impediments in the development of sonophoresis based TDD methods

There a number of inherent mechanisms in the sonophoresis based TDD based methods which affect their performances. These methods have been involved in a lot of branches in TDD research as discussed earlier. However, it seems that the sonophoresis research is still not very active if compared with other TDD methods. To illustrate this point clearly, we have carried out a time series analysis based on an autoregressive integrated moving average (ARIMA) model. We apply this analysis to illustrate the development of sonophoresis research over time. ARIMA is a common approach used for time series analysis of quantitative data and it can be used to determine trends of the data by evaluating the projection from past patterns. This approach can be particularly useful to model trends where the data have non-linear and fluctuating trends (such as the data in this work). ARIMA has been widely applied in social science areas to help people for making informed decisions. For example, the approach can help one (i) to find opportunities by analysing the market trends [52], (ii) to set right deposit rates in a bank by studying the money transaction data [53], (iii) to predict the traffic flow to help people to avoid traffic congestion [54]. At the moment, there is no such time series analysis for the trend of sonophoresis or MNs research. ARIMA uses univariate Box-Jerkins models which imply that only the past values of the variables are involved in the analysis and it does not consider data from other series [55]. The model consists of two parts: autoregressive (AR) and moving average (MA) which can be chosen together or individually depending on the modelled situation. The model applied for the purpose of this paper is MA (1) which means no AR method is employed. The number in the brackets indicates an order of the algorithm.

For the time series analysis, the data acquired from scientific paper database are differentiated twice with respect to time to achieve a stable model, which provides a time series constant for the data. In the present case, the estimated time series constant was 2.029. MA (1) model is then employed to fit the trend of sonophoresis publications per year (Fig. 3). The fit and forecast results using the MA (1) model are shown in Fig. 3. The predicted results on the number of publications in year 2020 are 60 which suggest a slight increment of 1.08% per year from the year 1970. Although the ultrasound technology is well understood, the results of this analysis shows that the development of sonophoresis is relatively slow and, there is no sudden increase or decrease in its interests as it may happen in many other methods or techniques. The number of publications on sonophoresis research has an averaged increment of 9.05% per year from 1970 to 2013. We believe there are two main reasons that have led to this slow growth as discussed below.

Firstly, the sonophoresis based TDD methods are not supported by well-developed theory. For instance, the current theoretical description of the ultrasound assisted molecular diffusion and convection is simply an extension of the hindered diffusion theory as shown in Equation (1) [56]:

$$\log P_{diff+conv}^{US} = \log \frac{Pe}{1-\exp(-Pe)} + \log\left(\frac{kT}{2z^2 F c_{ion} e_0} \frac{D_p^{\infty} H(\lambda_p)}{D_{ion}^{\infty} H(\lambda_{ion})}\right) + \log R \quad (1)$$

Where P is the drug permeability [m/s], Pe is the Peclet number [-], k is Boltzmann constant [J/K], T is the absolute temperature [K], z is the electrolyte valence [-], F is the Faraday constant [C/mol], c is the electrolyte molar concentration [mol/m^3], e is the electronic charge [C], D is the diffusion coefficient [m^2/s], H(λ) is the hindrance factor [-] and R is the skin resistivity [Ω·m]. The size and electrical properties of the molecules have been considered in

this model, but there is little or no theoretical development work on the effects of the modification to the skin due to cavitation effect which is the main factor that sonophoresis contributes to TDD. Well-developed theoretical descriptions of various mechanisms in sonophoresis based TDD are important not only for its further development and research but also for understanding the experimental data and combining ultrasound to other TDD methods.

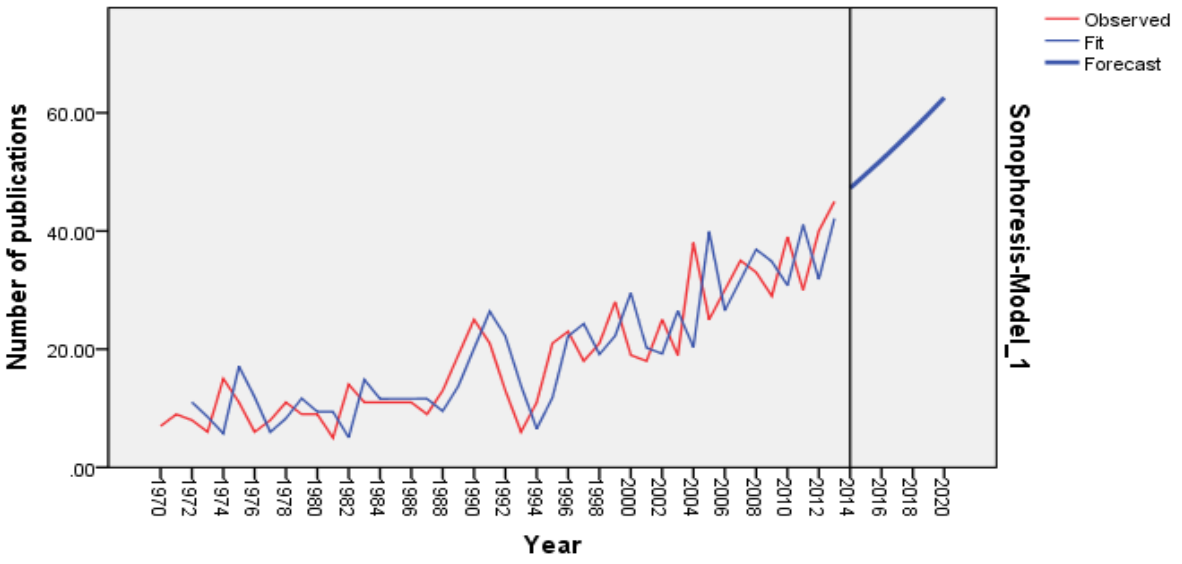

Fig. 3 Fit and forecast results of the trend of sonophoresis publications using MA (1) model developed in MATLAB (keywords for search: sonophoresis/phonophoresis using Scopus [42]).

Secondly, it seems that the researches on sonophoresis experiments involving drug permeability are somewhat chaotic and lack consistency. For example, the skin samples used in the experiments may lack a consistent quality and standard. Factors such as imperfections in skin samples, skin thickness variations, different sampling areas of the skin, different skin types, etc. also affect the drug permeability especially in the transport of large molecules. Furthermore, the uncertainties in the ultrasound system may alter the diffusion results. These uncertainties are included in the performance of the ultrasound system which needs to be

acquired using a hydrophone or force balance, and other details of the ultrasound setup (e.g., parameters such as distance of the ultrasound horn to skin surface and localized transport regions measurement [57]). Additionally, the significance of different mechanisms is not well understood and they must be determined. As mentioned earlier, sonophoresis includes several different mechanisms, all of which contribute to the molecular diffusion. The same ultrasound output, drug molecules and skin samples under different experimental conditions may show different diffusion results for ignoring some minor factor [58, 59]. Thermal effect, acoustic streaming and other phenomena can be significantly magnified due to those unnoticed factors varying the diffusion results. However, these factors are impossible to quantify separately in practice. Therefore, the overall progress of the research which particularly employs experimental methods seems to be slow. An illustration of percutaneous delivery of common drug molecules (MW are arranged from low to high) enhanced by sonophoresis is shown in Table 2. The table presents different diffusion results of a number of drug molecules. Even though all the experimental results have shown permeability increases, the data need to be carefully used in the context of sonophoresis. Therefore, the researchers have to ignore the less significant mechanisms in order to combine the sonophoresis to other TDD methods. To extend the combination range of sonophoresis and to increase the quality of combinations, sonophoresis experiments must be regulated and more elaborative experiments in consideration of the minor mechanisms must be executed.

Table 2. Ultrasound enhanced diffusion experiment data of selected compounds (mannitol, sucrose, cortisol, calcein, inulin, and insulin).

Solute property	Main experimental apparatus used	Skin type	Frequency (MHz)	Intensity (W/cm^2)	Exposure conditions	Composition of donor solution in experimental apparatus	Receiving solution in experimental apparatus	Analytical apparatus	Temp. (℃)	Data analysis	Result summary
Mannitol MW: 182 Da Log $K_{o/w}$ = -3.10	US transducer; Franz diffusion cell	*In vitro* hairless male Wistar rats skin 250-300g, aged 4-5 months	1.1	0.1 2.0	CW (5 min) pre-treatment	5% v/v [^{14}C] mannitol dissolved in 100ml ethanolic solution	24-33ml distilled water, 1.1ml aliquots were withdrawn every 30min within 5h	Liquid scintillation counter, thermocouple, a camera attached to a microscope (×125)	Receptor solution: 37	Mean± S.D. over 5h	Mean flux of radiolabeled [^{14}C] mannitol under US treatment of 0/0.1/2 W/cm^2 are: 90.51±19.51/not detectable/375.7±53.21 $pmol/cm^2/h$ respectively; Not detectable because mannitol is mainly mediated via transfollicular pathway, but under 0.1 W/cm^2 US the sebaceous sebum is released into hair follicle shaft. For histological results, see Fig. 3 in the original paper [60]
	Drugs applied to test sites followed by ultrasonic gel	*In vivo* on the upper back of hairless SD rat 200-300g	1.0	1.5	CW (3 min) pre-treatment	1µCi/µl D-[^{3}H]mannitol saturated with unlabelled D-mannitol in 20µl 90% ethanol and 10% water	Urine in bladder collected using catheterization every 15-30 min within 2h	Liquid scintillation counter	Room temperature	Mean± S.E.M. and student's t test, $P<0.05$ over 5h	Mean secretion rate of radiolabeled [^{3}H] mannitol shows 20-fold higher in the US treated group (n=4) than in the controls (n=12); for detailed data see Fig. 1 in the original paper [61]
	Modified Franz	Freshly excised	1.1	1.5	CW (20 min)	5µCi/ml [^{3}H]mannitol	15 ml 0.9% normal saline,	Liquid scintillation	Receptor solution: 29-	Mean± S.E.M. calculated over	Mean flux with and without US are 0.4±0.15 (n=4) and 0.5±0.15 (n=4) $pg/cm^2/h$, respectively. For

	diffusion cell; US transducer (90% efficiency)	surgical human skin *In vitro* hairless mice aged 6-7 weeks			pre-treatment	saturated in 3 ml 0.9% normal saline	500µl samples retrieved regularly	counter, light and electron microscopy	31, circulation Jacket: 32	24h	histological results see Fig. 1 and 2 in the original paper [62] Mean flux with and without US are 44.0±10.6 (n=5) and 27.1±5.5 (n=9) pg/cm^2/h, respectively. For histological results see Fig. 1 and 2 in the original paper [62]
Sucrose MW: 342 Da Log $K_{o/w}$ = -3.70	Franz diffusion cell, sonicatortest by a hydrophone, nylon mesh	Epidermis heat stripped from human cadaver skin	0.02	0.125	DC:10 Length 100ms (60 min) pre-treatment	1µCi/ml radiolabelled sucrose in a mixed solution (PBS 0.01mol/l, NaCl 0.137 mol/l)	15.8ml 0.01mol/l PBS mixed with 0.137mol/l NaCl	Thermocouple, scintillation counter, light microscope (×40)	Room temperature, no significant increasing during experiment (<2℃)	One value calculated at steady-state	The result is given by permeability $P=V\Delta C/(AC_d\tau)$, where V is the volume of the receiver compartment, A is the skin area (3.14 cm^2), ΔC is the measured concentration increase of the solution in the receiver compartment over a time period τ and C_d is the concentration of the solution in the donor compartment. The passive and sonophoretic permeability are 5.2×10^{-6} and 0.026 cm/hr, respectively. For histological results see Fig. 3 in the original paper [63]
	US transducer; Franz diffusion cell	*In vitro* hairless male Wistar rats skin 250-300g, aged 4-5 months	1.1	0.1 2.0	CW (5 min) pre-treatment	5% v/v [^{14}C]sucrose dissolved in 100ml ethanolic solution	24-33ml distilled water, 1.1ml aliquots were withdrawn every 30min within 5h	Liquid scintillation counter; thermocouple; a camera attached to a microscope (×125)	Receptor solution: 37	Mean± S.D. over 5h	Mean flux of radiolabeled [^{14}C] sucrose under US treatment of 0/0.1/2 W/cm^2 are 11.49±3.01/not detectable/51.36±2.62 pmol/cm^2/h, respectively. For histological results see Fig. 3 in the original paper [60]

Cortisol (Hydrocortisone) MW: 362 Da Log $K_{o/w}$ = 1.61	US transducer combined with iontophoresis devices (5mA pre-treatment for 20 min)	*In vivo* patients with unilateral carpal tunnel syndrome	1.0	0.5-0.8	DC: 20 Length 4ms (3-6min) pre-treatment	25 mg clinic use hydrocortisone acetate	n/a	Pain intensity rated on 10 levels called 10 points Visual Analog Scale (VAS)	Room temperature	Mean± S.D. Wilcoxon signed-ranks test, P<0.05	Group 1 (early stage of the disease), VAS before/after treatment: 7.4±0.5/1.8±1.9; Group 2 (moderate stage), VAS before/after treatment: 8.1±1.1/1.8±1.5; Group 3 (advanced disease stage), VAS before/after treatment: 8.0±1.2/4.2±1.9 [64]
	A 23-gauge butterfly catheter into a cubital fossa vein; US transducer	*In vivo* 16 human, aged between 18-33 ($\bar{X}$=25, SD=2.74)	1.0	1.0	CW (5 min)	30 ml aquasonic gel for control group; 30ml 10% hydrocortisone gel for experimental group	5 cc blood sample was drawn followed by 2 cc saline flush in different time point	Centrifuge; cortisol assay (0.45 µg/l sensitivity)	Body temp. & blood sample clotted at room temp.	Two-way ANOVA, P<0.05 over 30 min time period	The serum cortisol levels between US alone and hydrocortisone phonophoresis are: 1. 10 min before US treatment: 9.7±4.1&10.1±2.9 µg/dl; 2. Immediate after US treatment: 8.2±4.2&8.8±3.5 µg/dl; 3. 5 min after US treatment: 8.2±3.8&9.4±3.5 µg/dl; 4. 15min after US treatment: 7.6±3.6&8.0±.3 µg/dl. Four subjects reported intolerable heating [65]
	Modified Franz diffusion cell; US transducer (90% efficiency)	Freshly excised surgical human skin	1.1	1.5	CW (20 min)	6µCi/ml [^{3}H]hydrocortisone saturated in 3 ml 0.9% normal saline	15 ml 60% saline solution, 20% PEG 400 and 20% ethanol, 500µl samples retrieved regularly with equal	Liquid scintillation counter; light and electron microscopy	Receptor solution: 29-31, circulation Jacket: 32	Mean± S.E.M. calculated over 24h	Mean flux with and without US are 3.5±1.3 (n=5) and 3.0±0.6 (n=5) pg/cm^2/h, respectively. For histological results see Fig. 1 and 2 in the original paper [62]
		In vitro hairless mice aged									Mean flux over 24h with and without US are 46.8±4.6 (n=5) and 40.4±7.2 (n=5) pg/cm^2/h respectively. For histological results see Fig. 1 and

		6-7 weeks					compensation				2 in the original paper [62]
	Modified Franz diffusion cell; US transducer (10min recalibration /h);	*In vitro* intact Wistar rats skin 250-300g, aged 4-5 months, frozen in -20℃ up to 1 month	1.1 3.3	2.25 2.25	CW switched off for 10 min following each 50 min over 4h period	1μCi/μl [^{3}H] hydrocortisone dissolved in 100μl 5% ethanol solution, 1% v/v chemical enhancers (if needed) applied 1h before (azone or oleic acid)	5% aqueous ethanol, 1.1ml samples retrieved every 30min with equal compensation	Hydrophone; radiation force meter; thermocouple; liquid scintillation counter	Circulation Jacket: 28 temp. of skin surface see Fig. 3&4 in original paper	Mean± S.E.M. derived from linear regression analysis between 150-300 min	Mean flux: control/1.1MHz/3.3MHz are 0.0073±0.0105/0.0133±0.0016/0.0160±0.0052 pmol/cm^2/h respectively; Oleic acid/Oleic acid+1.1Mhz are 0.0494±0.0092/0.0583±0.0029 pmol/cm^2/h respectively; Azone/Azone+1.1MHz/Azone+3.3MHz are 0.0407±0.0054/0.1021±0.0125/0.0953±0.0172 pmol/cm^2/h respectively [66]
			3.3	0.75 2.25 2.25	CW or DC: 33.3 (Length 2ms interval as above over 4h period)						Mean flux of Azone-pretreated skin: CW 0.75W/CW 2.25W/pulsed 2.25W are 0.0388±0.0105/0.0378±0.0766/0.0540±0.0124 pmol/cm^2/h respectively [66]
	US transducer; Franz diffusion cell;	*In vitro* hairless male Wistar rats skin 250-300g, aged 4-5 months	1.1	0.1 2.0	CW (5 min)	5% v/v [^{3}H]hydrocortisone dissolved in 100ml ethanolic solution	24-33ml 5% v/v aqueous ethanol, 1.1ml aliquots were withdrawn every 30min within 5h	Liquid scintillation counter; thermocouple; a camera attached to a microscope (×125)	Receptor solution: 37	Mean±S.D. & t test, P<0.0208 over 5h	Mean flux of radiolabeled [^{3}H]hydrocortisone under US treatment of 0/0.1/2 W/cm^2 are: 0.105±0.023/0.0478±0.006/0.81±0.14 pmol/cm^2/h respectively. For histological results see Fig. 3 in the original paper [60]

Calcein MW: 623 Da Log $K_{o/w}$= 1.56	Sonicator; Custom-made vertical glass diffusion cells	*In vitro* full-thickness skin from porcine ears	0.02	15	DC:10 (2 hours)	200mg calcein in 11ml PBS at pH 7.4	38 ml PBS at pH 7.4	Confocal microscope	Room temperature	n/a	There is no quantitative data in this research. However, it is using cross-sectional view of the skin (confocal images) to show the permeation of calcein with/without ultrasound treatment for 2 hours duration within a 20 µm depth. It suggests that some areas showed great increment of permeation of calcein after the ultrasound treatment while some areas did not [67]
	Three separate US Systems; diffusion cells	*In vitro* back and flank skin of female Yorkshire pigs	0.02 0.04 0.06	7.5	DC:50 (length 5s)	0.2% w/v calcein in 2.5ml PBS	12 ml PBS, sampled at 2 h intervals between 18 and 26 h	UV–visible spectrophotometer absorbance wavelength: 494 nm	Room temperature 25°C	Mean± S.D. over 8 hours	The ultrasound treatment time varies according to when the electrical currents reach 225/275/335 µA. The results show that permeability is not affected by either electrical resistance or frequency change. The scales of permeability for passive/LTRs/non-LTRs/total are $1\times10^{-6}/10^{-2}/10^{-5}/10^{-3}$ cm/h, respectively [68]
	custom-built US transducer; diffusion cells	*In vitro* male WBN/ILS-Ht strain hairless rats	0.041	0.06 0.12 0.3	CW (2 hours)	1mM calcein in 10ml PBS at pH 7.4	22 ml PBS, Sampled at 30 min intervals between 12 and 14 h	Spectrofluorometry excitation wavelengths: 488 nm	Room temperature	Mean± S.D. over 2 hours	After 12 hours of passive diffusion, ultrasound is in turn applied for 30min to each at 0.06, 0.12, 0.3, 0.06 W/cm^2 for a total 2 hours. The flux increments from a base 1.1×10^{-2} nmol/cm^2/h are 120, 8900, 23000,5100 folds, respectively [69]
Inulin MW: 5.0k Da	Drugs applied to test sites followed by ultrasonic gel;	*In vivo* on the upper back of hairless	1.0	3	DC: 80 (5 min)	0.22µCi/µl [^{3}H]inulin saturated with unlabelled	Urine in bladder collected using catheterization every 15-30 min	Liquid scintillation counter	Room temperature	Mean± S.E.M. & Student's t test, P<0.05 over 2 hours	Mean secretion rate of radiolabeled [^{3}H]inulin shows 5 times higher in the US treated group (n=4) than in the controls (n=4), for detailed data see Fig. 2 in the original paper [61]

		SD rat 200-300g				inulin in warm water	within 2h				
	Flanged glass cylinder glued on the rat's shaved lateral flank	*In vivo* Sprague Dawley rat of either sex, PBS hydrated for 1 h	0.02	7	DC: 50 length 5s (2 min)	10 μCi/mL radiolabeled inulin in 2ml PBS	Urine in bladder measured every 30min for 5h			Means only, no SD data available, 5 hours	Mean secretion rate of radiolabeled inulin before/after the ultrasound treatment are 7.4×10^{-6} cm/h and 1.5×10^{-4} cm/h, respectively [70]
Insulin MW: 5.8k Da	Flanged plastic cylinder was glued to animal's abdomenand sonicator	*In vivo* hairless rats 280±20g, aged 8-10 weeks	0.02	2.5 5.0 10.0	DC: 10 length 0.1s (60 min)	3ml of insulin solution at a concentration of 100 U/ml	0.6ml blood samples from jugular vein were immediately centrifuged and the serum was collected	Thermocouple; biochemistry analyser (using glucose oxidase method)	Donor solution: 27, animals' body temperature: 38	One-factor ANOVA analysis, P<0.05 over 60 min	Blood glucose level of control group is 12.60±2.07 mmol/l, the initial and after US treatment for 2.5W, 5.0W, 10.0W are: 12.91±1.68&11.22±1.71/12.20±0.60&10.65±6.22/ 12.33±0.67&7.46±2.95 mmol/l, respectively [71]
				2.5	DC: 10/20/30 length 1.6s (60 min)						Blood glucose level of control group is 12.60±2.07 mmol/l, the initial and after US treatment for DC=10/20/30 are: 12.91±1.68&11.22±1.71/11.84±1.38&9.79±2.89/1 0.62±0.88&4.91±2.77 mmol/l respectively [71]
				2.5	DC: 40					One-factor	Blood glucose level of control group is 12.60±2.07

					length 0.2/1.6/3.2s (15 min)					ANOVA analysis, P<0.05 over 15 min	mmol/l, the initial and after US treatment for pulse length=0.2/1.6/3.2 are: 12.46±0.63&11.56±1.95/12.38±0.67&6.43±3.16/13.41±1.23&5.11±1.45 mmol/l respectively [71]
	1 mm thick water tight standoff arranged between the axillary area of the pig and the array	*In vivo* Yorkshire pigs (100–140 lbs)		0.1	DC: 20 Length 200ms (60 min)	100 U/ml insulin filled in 1 mm thick water-tight standoff	0.3 ml blood samples from the ear vein every 15 min for 90 min	Blood glucose monitoring System	Room temperature	One-factor ANOVA analysis, P<0.05 for both 60 and 90 min	Blood glucose level before experiment is 146±13 mg/dl (n=6); Control group increased 31±21 mg/dl (n=3) after 90 min; US treatment group decreased 71±5 mg/dl (n=3) from beginning at 60 min and decreased 91±23 mg/dl (n=3) from beginning at 90 min [72]

Note: CW = continuous wave; DC = duty cycle; PBS = phosphate buffered saline; S.E.M. = standard error of the mean; SD = standard deviation.

2.2 Thermal effect of sonophoresis

The most obvious phenomenon during the sonophoresis treatment is the thermal effect and it is particularly relevant when using high frequency. The absorption of the sound in skin increases as the ultrasound frequency goes up, which means that the energy would be stored in skin rather than transmit through [73, 74]. The rise in the temperature of the skin increases the kinetic energy of the drug molecules which have a positive effect on the drug diffusion rate. However, the intensity in the application of sonophoresis is usually low; therefore, the thermal effects on the kinetics energy of the drug molecule and hence the drug permeability is not significant. For example, when 1.5 W/cm^2 ultrasound was applied on hairless rat, the temperature change is found to be only around 1-2^{o}C [61]. If the temperature increases significantly due to ultrasound treatment, it can cause skin injury. In particular, it has been reported that when the temperature reaches 43°C and maintains this temperature for 60 minutes or longer, the cellular reproduction may be restrained [75]. Although the thermal effect generated by ultrasound is the most basic phenomenon due to energy gain/loss, it has the potential to increase and control the drug diffusion rate when combined with MNs, particularly, dissolving MNs. We discuss this point further in section 4.

2.3 Convection in acoustic streaming

Another ultrasound related phenomenon is termed as acoustic streaming which is a kind of fluid flow driven by the pressure gradient and generated by acoustic field [76]. The permeability enhancement due to acoustic streaming is hard to define but its importance is realized by the scientific community [77]. Different effects of the acoustic streaming can be identified by Reynolds number of the flow. With low Reynolds number, Lighthill [78] has described the relationship between the net force of unit volume $\mathbf{F}_j$ [N/m^3] and forces that generated by the momentum flux $\rho_0 \mathbf{u}_i \mathbf{u}_j$ (Reynolds stress) [N/m^2], the pressure $\mathbf{p}$ [N/m^2] and the viscosity μ [N·s/m^2]. The mathematical definition is given:

$$\boldsymbol{F}_j = \rho_0 \left(\overline{\boldsymbol{u}}_i \frac{\partial \overline{\boldsymbol{u}}_j}{\partial x_i} \right) + \frac{\partial \overline{\boldsymbol{p}}}{\partial x_j} - \mu \nabla^2 \overline{\boldsymbol{u}}_j \tag{2}$$

Where ρ_0 is the density of the volume of the fluid [kg/m^3], and **u** is the velocity vector [m/s]. As evident from the first two terms on the right hand side, the Reynolds stress and pressure gradient affect the net force in the tissue positively (the term in left hand side of the equation). But, as shown by the third term on the right hand side, higher fluid viscosity results in lower net force which indicates that the ultrasound field can apply higher forces on the flow in a less viscous solution. Tachibana [79] shows that the diffusion of lidocaine under the same ultrasound condition is higher when it is in the aqueous formulation instead of a gel. Therefore, the permeability enhancement caused by ultrasound generated force was experimentally proven. The phenomenon was successfully extended by applying this mechanism on highly aqueous tissues. Lewis [80] applied 1.58 MHz ultrasound on brain and avian muscle tissues, and report that the enhancements of Evans blue dye are 5.6 fold and 2.2 folds, respectively. Using the experimental set up in Fig. 4, Cheung [81] has demonstrated that under 3 MHz ultrasound treatment the permeability of bovine serum albumin (BSA) increases 1.6 fold in intrascleral delivery.

Although the researches on using acoustic streaming at low Reynolds numbers have shown increment on diffusion rate in soft tissues, the permeability enhancement on viable epidermis and dermis is still relatively unknown. As well known, viable epidermis and dermis of the skin are the most relevant skin layers for MN application as the MNs pierce the SC and deliver the drugs in the skin layers below the SC. However, it is logical to state that there are good potential for combining ultrasound with solid or hollow MNs because the MNs can create channels that reach viable epidermis. As such the ultrasound may reach the lower layers of the skin and help increase drug permeability in these skin layers. This combination

of TDD methods should be able to provide higher diffusion rate in comparison to sole MNs. There are some other mechanisms caused by acoustic streaming at high Reynolds numbers which are also important. These mechanisms will be discussed in sections 2.4-2.6.

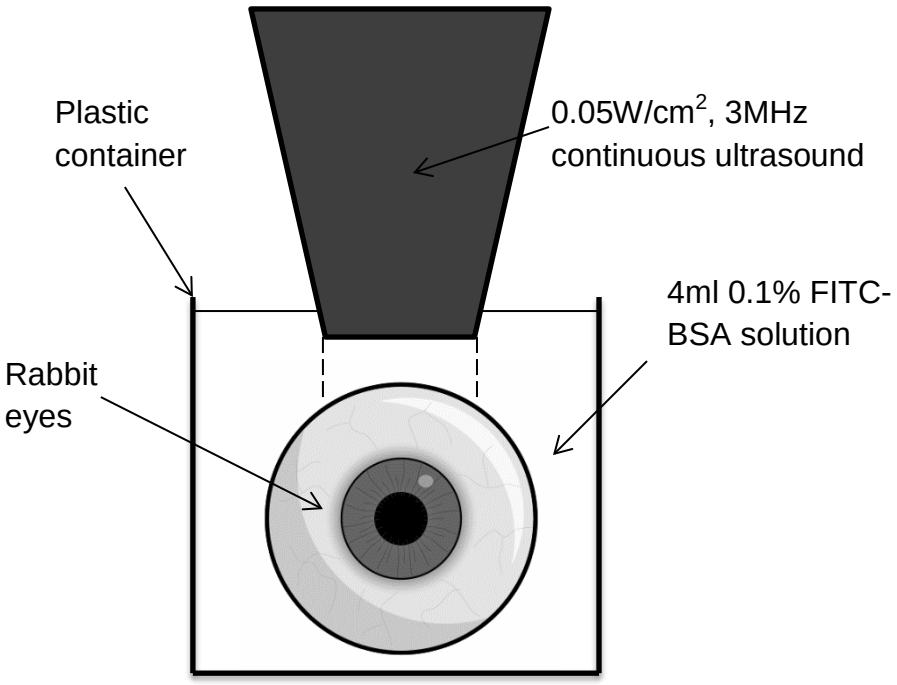

Fig. 4 The setup of ultrasound enhanced intrascleral delivery

2.4 Acoustic cavitation in high frequency sonophoresis

Acoustic streaming can produce different mechanisms in sonophoresis. The most important mechanism is acoustic cavitation (bubbles) which is generated in the liquid within or out of the skin. When ultrasound waves keep compressing and tensing the liquid in the tissue, the local pressure of liquid falls below vapour pressure, and therefore, the cavitation occurs. The cavitation can be divided into two types: stable and inertial discriminated by how long can the bubbles survive [82]. Frequency, intensity and duty cycles are used to control the cavitation types to achieve different applications. The cavitation generated during high frequency ultrasound treatment is much smaller in size as compared to those from the use of low frequency ultrasound. The relationships between the frequency and the bubble radius are presented by Gaertner [83]:

$$f(\emptyset) = 1/\left\{\sin^3 \emptyset\,[-(\sin^4 \emptyset + \sin^2 \emptyset)(pR_0 + 3\alpha) - pR_0]^{\frac{1}{2}}\right\} \tag{3}$$

$$\emptyset = \arcsin(R_0/R)^{\frac{1}{2}} \tag{4}$$

Where f is the frequency of the ultrasound [Hz], R_0 is the nucleus radius [m], α (surface tension) is a constant related to the medium [N/m] and R is the radius of the bubble [m] under pressure p [N/m^2]. The equation indicates that the ultrasound frequency f is inversely related to the radius of the bubble R. The equation has also suggests that cavitation could be generated inside the skin or simply within the SC layer if the frequency is high enough

2.5 Rectified diffusion

Due to the relationship between frequency and bubble radius, the most important phenomena in high frequency sonophoresis, which is called rectified diffusion (Fig. 5), has been revealed by Blake [85]. The mechanism can be explained as follows. On the positive pressure half-cycle (the local pressure increased under the ultrasound field) the gas in a small bubble will be compressed, the shell becomes thicker as a result the concentration gradient decreases because the bubble absorbs more drugs from surrounding environment. Some gas then diffuses outwards from the core of the bubble into the liquid. On the contrary, during the negative half-cycle of pressure, the surface of the bubble is expanded which makes it much larger than the compressed bubble. However, these two rates are not equal as the surface area of the bubble is greater during the negative (tension) half-cycle, and as diffusion rates are proportional to the exposed area, the bubble must gain some gas over a complete cycle [86].

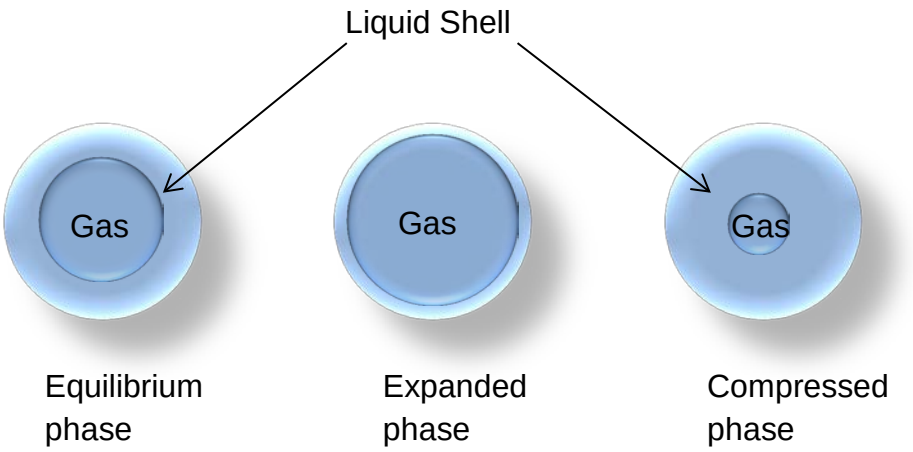

Fig. 5 A schematic diagram of rectified diffusion [87]

In the process of the rectified diffusion, the bubbles generated by cavitation are pushed by the Bjerknes force under acoustic pressure gradient to move downward [88]. The Bjerknes force is generated under the acoustic field which directly increases the diffusion rate of the drug molecules. It can then push the bubbles forward, thereby, increasing the diffusion rate. The basic expression of Bjerknes force is shown as:

$$F = -\langle V \nabla p \rangle \tag{5}$$

Where < > denotes the average over one acoustic period, V is the volume of the bubble and p is the acoustic pressure. This equation can be further extended to define the Bjerknes force at any location **r** and time point t [89]:

$$\boldsymbol{F_{Bi}} = -G_i(\mathbf{r})\frac{1}{T}\int_0^T V(\mathbf{r},t)\cos[\omega t + \psi_i(\mathbf{r})]dt \tag{6}$$

Where T is the acoustic period, V(**r**,t) is the instantaneous volume of the bubble, G(**r**) and ψ(**r**) are pressure and phase gradients, respectively. Because the rectified diffusion is the dominated effect in high frequency sonophoresis, the bubbles must be small enough to survive inside the skin. This means that the diffusion of molecules may not necessarily increase. Bommannan et al. [90] have reported that after 20 min ultrasound treatment (2 MHz,

0.2W/cm^2), the diffusion of salicylic acid (138.12 Da) is not increased. However, the diffusion rate is 4 times higher at 10 MHz 0.2W/cm^2 when the bubble size is small enough to move inside the skin. But, if the size of the bubbles is further reduced, the diffusion rate is dropped to 2.5 fold at frequency of 16 MHz and power of 0.2W/cm^2. Therefore, properly selected frequency will significantly increase the diffusion rate. This provides a great potential to deliver medium-sized or large molecules by using the microneedles and ultrasound combination.

2.6 Acoustic cavitation in low frequency sonophoresis

The low frequency sonophoresis generally refers to 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 [50]. Researches showed 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). Mitragotri et al. [63] have shown that the enhancement ratios induced by low-frequency ultrasound (20 KHz) is up to 1000-fold than that induced by therapeutic ultrasound (1 MHz) on butanol (74.12 Da) and sucrose (342.29 Da). This is due to the fact that inertial cavitation becomes the dominated mechanism and it can directly disrupt the SC layer to increase the permeability. The application of low-frequency ultrasound can be divided into two forms: simultaneous sonophoresis (decreased after ultrasound is turned off) and pre-treatment sonophoresis (remains in highly permeable state for several hours). The mechanisms of both of these two methods rely on inertial cavitation, i.e., bubbles generated within the coupling medium by ultrasound and would grow and collapse violently. The difference between them is the pre-treatment sonophoresis and generates more bubbles to change the structure of the *stratum*

corneum (about 30% of the lipids layer is removed by micro-jet [91]) while the simultaneous sonophoresis only intends to increase the porosity.

As mentioned before, the main type of cavitation which helps in permeability increment is the inertial cavitation [92]. It occurs due to pressure variations induced by ultrasound, resulting in rapid growth and collapse of bubbles formed in the coupling medium. The collapsing of the aforementioned bubbles in a spherically symmetric environment results in the release of a shockwave causing structural changes of the surrounding tissue. The suddenly raised pressure gradient conducted by the shockwave spreads evenly on the skin surface. Therefore, it can only produce limited damage. However, the skin surface will cause the bubbles involutes asymmetrically which results the bubbles collapse from the top surface [93]. Water is then forced into the bubbles and a high-speed micro-jet will be developed from the top surface. The power generated by the micro-jets transmits downward and is focused on a tiny spot of skin surface. These micro-jets can severely disrupt the SC layer and have been confirmed as the main contribution to the permeability increment [94]. The mechanism of forming micro-jets is shown in Fig. 6.

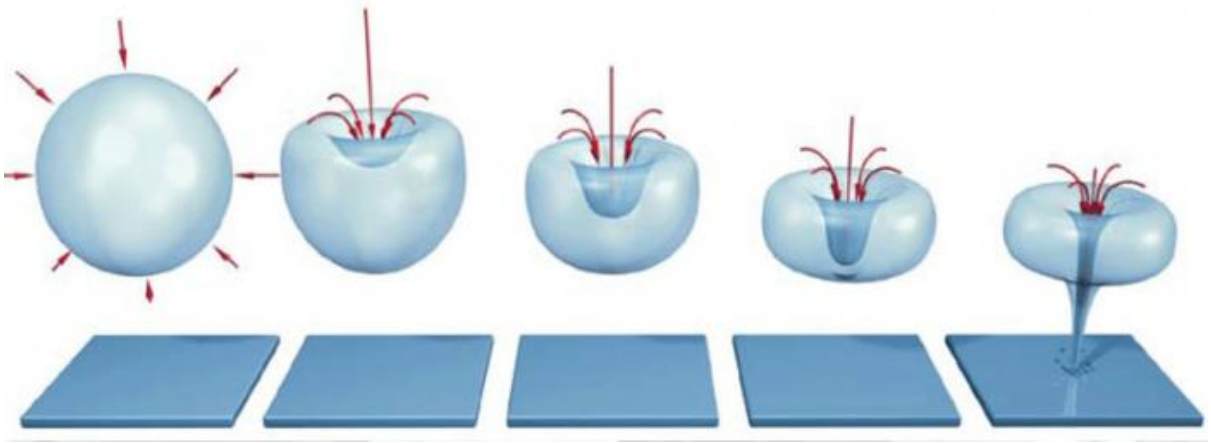

Fig. 6 The mechanism of cavitation collapse near skin surface which creates a micro-jet.

Because of the huge bubble size in low frequency sonophoresis, the main effect is based on the microjet that occurs during bubbles collapse. The permeability can remain high for hours after a short ultrasound application. Therefore, a relatively high input power must be employed to create channels on the *stratum corneum* otherwise the permeability cannot be maintained. Fig. 7 indicates that insufficient power of inertial cavitation do not create significant pathway on the porcine *stratum corneum* by micro-jet.

The inertial cavitation has been studied by many researchers. It shows excellent potential when combined with solid MNs (discussed in section 4). Solid MNs can provide opportunities for the inertial cavitation to physically contact the viable epidermis. It will greatly reduce the pretreatment time and keep the permeability increased for a longer period of time. These issues are discussed in detail in the following sections.

2.7 Safety of ultrasound application

The tolerant limit of skin to the ultrasound is an important issue for the applications of sonophoresis. However, only a few studies have been conducted in this area and it seems that this technology is still underdeveloped and has a long way to go before clinical trials. An *in vitro* study on human skin shows that at 2.5W/cm^2, the skin structure modification can be identified under electron microscopy [70]. Although these is no *in vivo* study on human, ultrasound has been applied on canine subjects and urticarial reaction is found when the power reaches 16W [95]. Currently, the intensity of most sonophoresis applications are under 3.5W/cm^2 [96], so that the safety can be assured.

3. Microneedles (MNs) in TDD

The MNs research has been carried out for over 40 years now and it is one of the most promising technologies among the TDD methods. MNs are a technology developed from transdermal patches and hypodermic needles, attempting to gain advantages and eliminate disadvantages from both. The idea of MNs comes from the patent of Gerstel and Place [97] in the early 1970s when they introduced the concept to make micropores in the skin. In the 1990s, the microchip fabrication technology provided the new way to make longer three-dimensional microstructures of silicon and mass production of microfabrication tools so that the experimental demonstrations can be made. The first study of using MNs to enhance TDD process was devoted in 1998 [98]. Following this work, MNs technology has been developed rapidly and greatly extended in pharmaceutical area. Compared to hypodermic needle, MNs are painless and can significantly reduce the pain depending on the length of the needle. Gill et al. [99] have used different length of MNs from 480 µm to 1450 µm tested on human volunteers and found out that the needle length below 750 µm is painless and bloodless. However, the MNs that are less than 300 µm long have been shown not able to penetrate the skin [100]. The main development of the MNs technology which makes it distinct from other TDD methods is that it has greatly extended the range of drug molecular weight that can be delivered. Verbaan [101] used 700 µm MNs array to successfully deliver fluoresceinisothiocyanate coupled dextran which has molecular weight of 72 kDa. Influenza vaccine has also been delivered by using biodegradable MNs on mice [102].

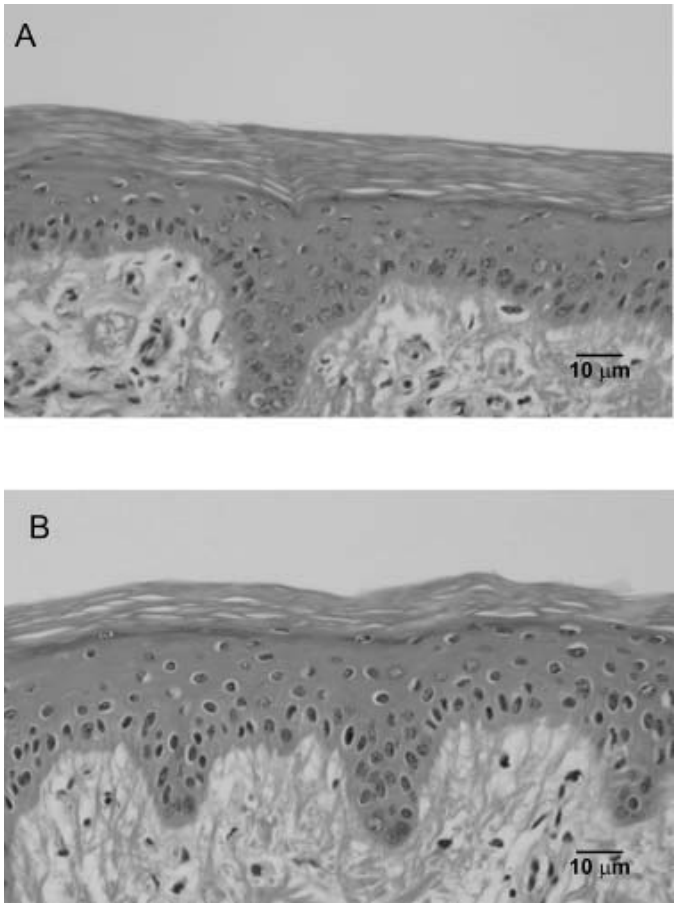

Fig. 7 The cavitation generated by micro-jet applied on the porcine *stratum corneum*. A) No ultrasound applied on the skin; B) 20 kHz, 2.4 W/cm^2 ultrasound applied on the skin for 10 min **[103]**

The MN research has developed strong activities in the last decade as indicated by a large number of publications. The average increment rate of MNs publications is 77.9% per year for the past 10 years (2004-2013) while the overall increment rate is 14.9% since 1970. To have a better understanding on the developing future of MNs, an ARIMA model is employed again which provides a rough forecast on the trend of MNs research. The data for the number of publications have been differenced twice to acquire a relatively stable model. The algorithm orders that are suitable for this model are chosen by considering the trend of autocorrelation coefficient and partial autocorrelation coefficient calculated from the model. As a result, the AR (1) and MA (1) models have been confirmed as the optimum choice with

minimum deviation from the original data (mean absolute percent error 139.8%). The trend of MNs research until 2020 is then forecasted. After recognizing the pattern of the past 40 years, the ARIMA model suggests that the number of publications will reach 694 in 2020 in comparison to 446 in 2013 which gives a 99.2% increment rate per year. The observed, fitted model and forecasted data have been shown in Fig.8 which suggests that in comparison to the trend in the past 10 years, the research in the MN area will become more active in the coming years.

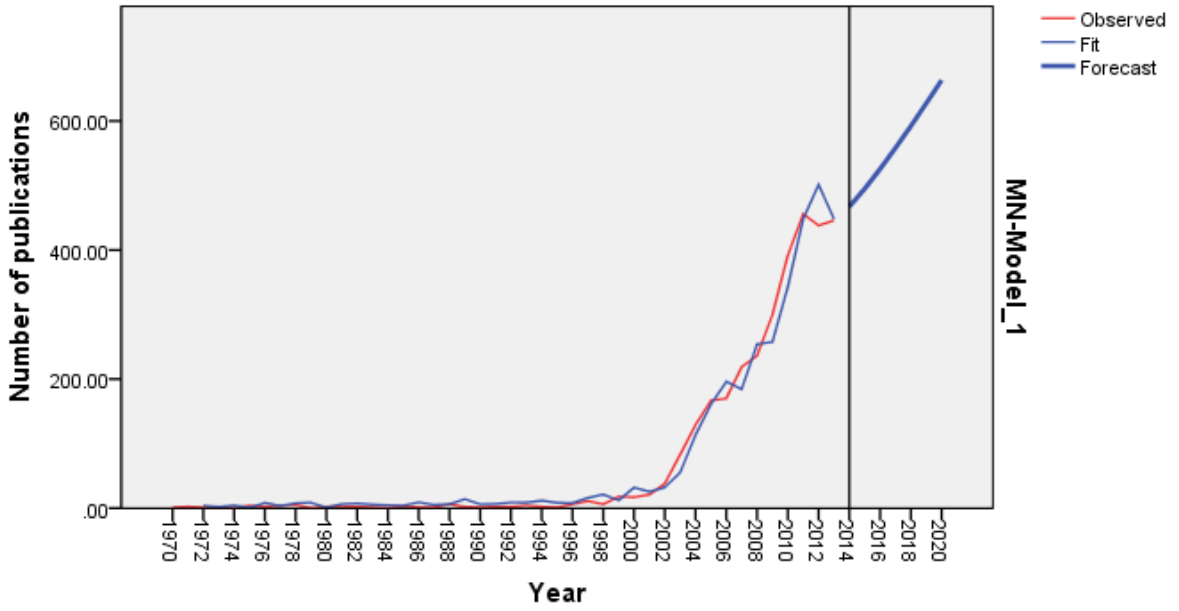

Fig. 8 The observed, fitted model and forecasted trend of MNs research presents from 1970 to 2020 using ARIMA model

There are a number of review papers focused on MNs related areas other than the mechanisms of MNs, for example fabrication techniques of MNs [104], permeability enhancement of MNs [105] and ethical study of MNs [106] etc. However, this review paper intends to explore the potential of MNs combined with sonophoresis. Therefore only a brief discussion on MNs is presented. Conventionally, MNs is divided into two different types: solid MNs and hollow MNs and their main mechanisms are shown in Fig. 9.

This classification method can only present the basic idea of the delivery process. The real delivery efficiency depends on many other factors. Martanto et al. [107] performed experiment to reveal the relationship between the MNs insertion depth and force. They also found out the real insertion depth is much lesser than the length of the needles due to the skin buckling. Their histological results shows the real penetration is only 100-300 µm by applying 1080 µm length MNs.

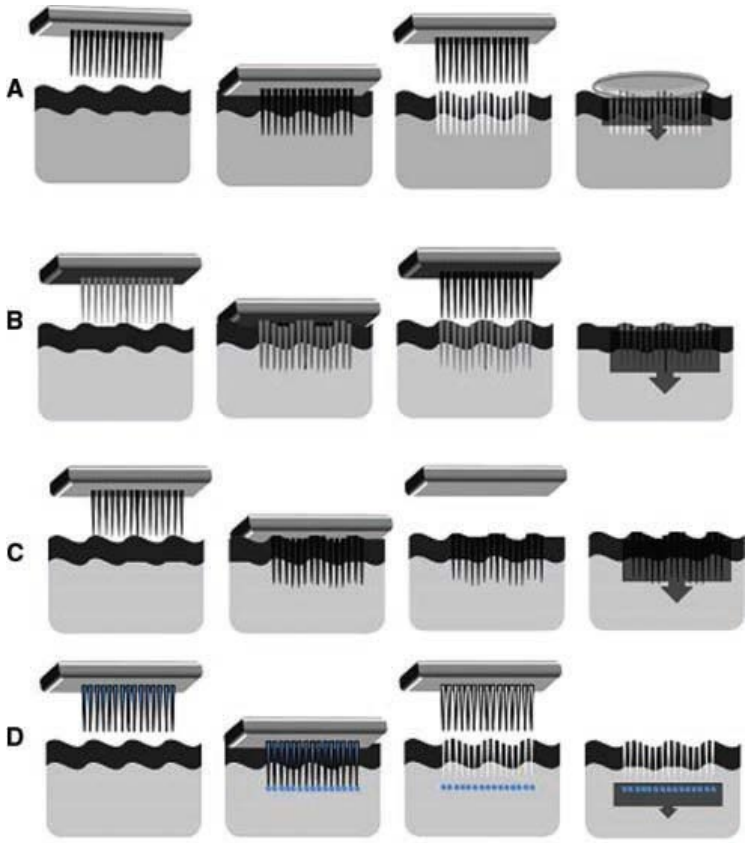

Fig. 9 Main mechanisms of applying MNs: A) Solid MNs using poke with patch method; B) Solid MNs using coat and poke method; C) The Dissolving MNs and D) Hollow MNs for liquid delivery **[108]**

The geometry of MNs has been proved to be another important parameter that can directly affect the diffusion results [109]. Parameters such as tip radius, density, distance between needles etc. are all brought to the consideration for being the important factors in MNs patch

design [110]. Besides these factors, the main purpose of MNs enhanced TDD is to reduce the resistance of the skin. Therefore, high force has been applied to reduce the skin buckling effect so that it can increase the real insertion depth [111]. Super short MNs with 70-80 µm length are also introduced to increase the permeability [112]. This kind of MNs is not aimed at penetrating the skin but to scrape the skin surface in order to lessen the thickness of SC layer.

3.1 Solid microneedls

The solid MNs are stiff and steady in structure and deliver drug by coated drugs on their surface or micro conduits created on epidermis to let drugs go through it. The methods of solid MNs delivery can be done in four different ways:

(1) The poke with patch approach was proposed by Henry et al [98] who used solid MNs to penetrate human cadaver skin and reported that the permeability of calcein increased by up to three orders of magnitude. The mechanism of poke with patch method involves the use of solid MNs to pierce the *stratum corneum* first and then put a patch during the insertion or immediately after removal of the needles. An image is shown in Fig. 10 to illustrate this point.

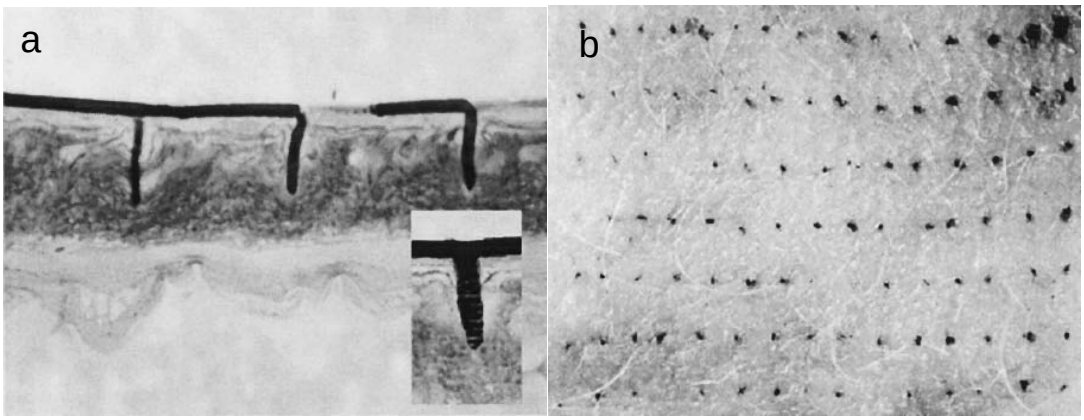

Fig. 10 a) Solid MNs penetrated into the skin b) after the MNs are removed, the treated skin is ready for applying the drug loaded patch **[101]**

Research done by Wermeling et al [113] indicated that Naltrexone (used to treat opiate and alcohol addiction) only takes two hours to reach the steady state plasma concentration with MNs pre-treatment and last for 48 hours while the transdermal patch cannot give any detectable results over 72 hours. Martanto et al [114] compared the hypodermic needle with the solid MNs (105 needles) by injecting insulin to diabetic rats and reported positive results on delivering large MW proteins.

(2) The coat and poke approach is used to coat the drug on the surface of the MNs which is then applied on the skin (Fig. 11). Large molecules such as human growth hormone (22 kDa) and ovalbumin (45 kDa) can be transported through skin [115, 116] in this way.

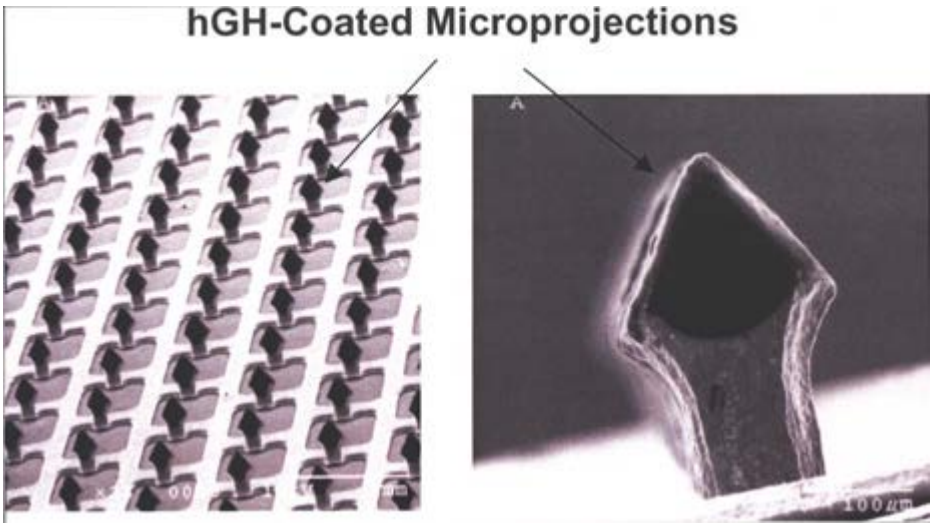

Fig. 11 Solid MNs coated with human growth hormone **[115]**

The main advantage of this method is that the MNs can retain its mechanical strength while delivering drugs. Therefore, the permeability loss during the skin recovery can be avoided [117]. However, the drug reloaded during the delivery process can be a problem. The most efficient way is to put the matrix of MNs on a roller with coating device on one side. So the MNs can maintain in inserting and coating rotation when rolling on the skin surface. But this

method cannot increase the diffusion rate after the drug is injected which makes it faces the same limitation with the poke with patch approach.

(3) The dip and scrape method employs the array of MNs coated with drugs to treat and then scrape multiple times across skin to create micro-abrasions. These micro-enhancers have proved that they can effectively breach the skin barrier and increase the permeability of the drug molecules [118].

(4) The dissolving MNs are made from a polymeric material and will release drugs after the MNs dissolves within skin (see Fig. 12 for an example). The main problem in this kind of MNs is the drug mutations during the high temperature moulding and fabrication [119]. A novel dissolving MNs introduced silk fibroin as the base of the MNs because it is rigid in structure, friendly to donor permeates and dissolving very quickly [120]. More recently, biopolymers have been used in the fabrications of dissolving microneedles because they can reduce the cost of material and also increase the biocompatibility of the products [121].

3.2 Hollow MNs

The hollow MNs have similar mechanism with hypodermic needles but they are much smaller in size (e.g., 500 μm in length). The drugs can continuously flow into the skin through the hollow capillaries but in a low transmission rate. If the pressure is high the drug would overflow through the bypass between needle and skin to the atmosphere. One significant advantage of the hollow MNs among other types is that it can extract a small amount of blood sample underneath the skin which enables monitoring on quantities of body fluid, for example, blood glucose level [122]. This technology is then further developed by optimising the geometry and arrangement of the patch so it can give reasonable extraction rate [123]. Due to the small size of the hollow MNs which increase the difficulty of the penetration and injection, works also have been done on how to optimise the process of MNs

insertion and injection pressure during drug delivery. Martanto et al [107] demonstrated that a little retraction during injection process can significantly increase the fluid infusion because it releases the compaction of the skin. The flow rate inside the hollow needles is another parameter that has been studied and optimised [124]. Besides the above, the stiffness of the MNs, difficulties in fabrication and cost are very important issues in the development of hollow MNs [125].

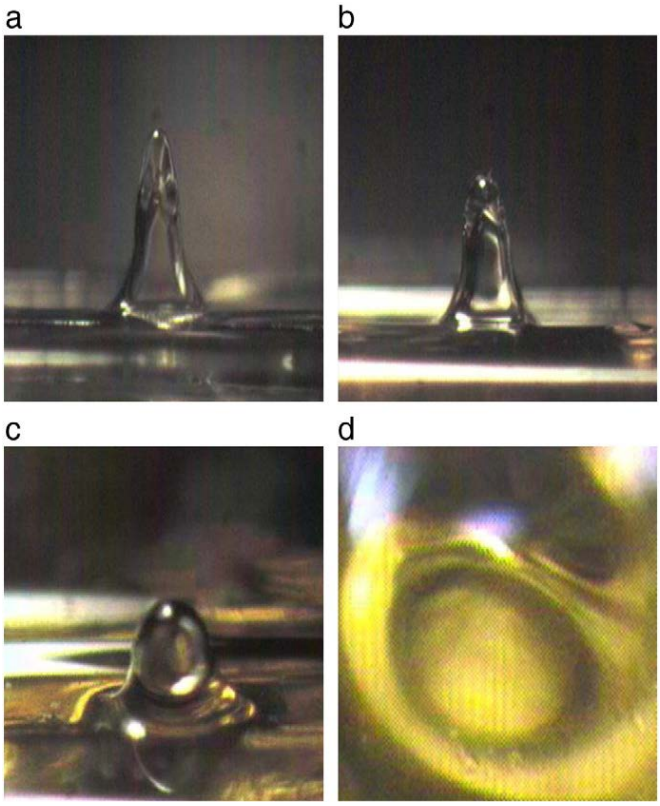

Fig. 12 The process of dissolving fibroin MNs in the porcine skin after time a) 0s, b) 15s, c) 30s and d) 60s **[120]**

4. Potential of combining MNs and sonophoresis

This review paper focuses on discussing the ultrasound and MNs combination as a TDD method for the future. Therefore, we devote this section of this paper to discuss this point in detail. As discussed in the paper before, research on MNs and ultrasound are both actively

pursued in recent years, which involve different mechanisms for drug delivery and transport. Therefore, the main point of discussion in this section is how to combine these mechanisms. In addressing this issue, we discuss the different possible ways and benefits of combining MNs and ultrasound.

We can use hollow MNs to pierce the skin and apply ultrasound simultaneously, where the hollow MNs should be under the ultrasound field. The hollow MNs are able to provide certain permeability increment while the simultaneous ultrasound field can enhance the flow rate via convection (this combination can be referred to the categories (iv) and (v) in Fig. 1). In fact, there are already some attempts to combine these two mechanisms. For example, a Singaporean research group has attempted this combination to deliver calcein and BSA [126]. They have used 100 µm long hollow MNs (80 µm outer diameter at the base) to pierce the skin and have attached an ultrasound transducer at the back of the MNs patch. The ultrasound output parameters have consisted of 20 kHz frequency, 0.5W/cm^2 intensity and 20% duty cycles (length 10µs) which can maintain the temperature stable at 37°C. The enhancement of this specific circumstance shows 9 times higher for calcein and 12 times higher for BSA in compare to passive diffusion, respectively, as shown in Fig. 13.

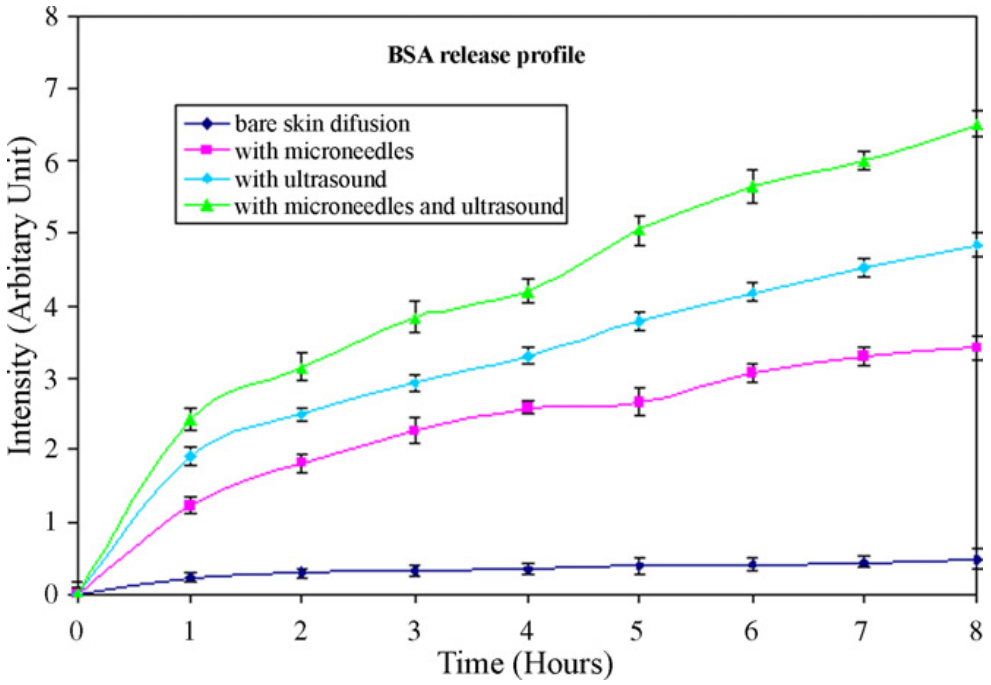

Fig. 13 BSA release profile using simultaneous ultrasound combined with hollow MNs [126]

We can also use solid MNs combine with ultrasound cavitation. The solid MNs can create visible holes that will provide permeability increment. If we apply high intensity ultrasound on the MNs pretreated base, the ultrasound cavitation will be able to produce more significant enhancement than on its own. This is because the ultrasound cavitation can contact the underneath layers of the skin where these bubbles can release more potentials. This combination is especially efficient in the delivery of large molecules and it can be a good preparation for a long-term TDD (this combination can be referred to the categories (iii) and (iv) in Fig. 1). A recent paper which has used this kind of combination to delivery BSA through skin is reported by Han and Das [16]. Two sets of solid MNs with length 1.2 mm and 1.5 mm were applied for 10 min to create pores and disrupt the skin surface. The 20 kHz, 9-18W ultrasound is then mounted to create inertial cavitation for another 10min. The permeability increment results of the combination in comparison with passive diffusion, ultrasound only and MNs only are shown in Fig.14.

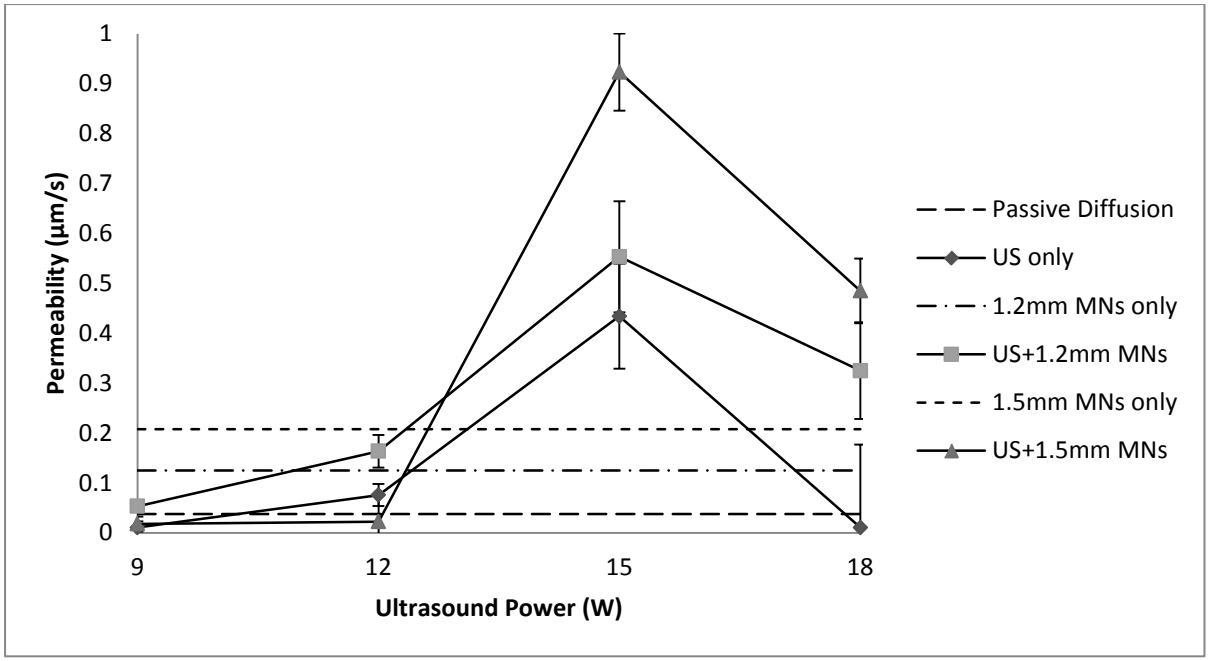

Fig. 14 BSA permeability results of passive diffusion, ultrasound only, MNs only and MNs combined with ultrasound.

It may also be possible to use solid MNs to pretreat the skin and then apply ultrasound simultaneously. This combination can be specialized to deliver medium size molecules. The difficulty in the delivery of medium size molecules is the low diffusion rate after the molecules have entered the viable epidermis. Solid MNs are sufficient to deliver the medium size molecules to the underlying layer. The simultaneous ultrasound can then apply pressure gradient on the molecules and achieve diffusion increment via rectified diffusion (this combination can be referred to the categories (iv) and (v) in Fig. 1).

Furthermore, we can use dissolving MNs to pierce the skin and then apply low intensity simultaneous ultrasound to active the maximum thermal effect. The dissolving MNs can pierce into skin and release drug at a constant rate according to the local temperature. Ultrasound is able to provide relatively accurate local temperature adjustment. It can provide

temperature modification thereby controlling the drug release rate. If this combination can be well developed, it will achieve both drug delivery rate increment and control (this combination can be referred to the categories (ii) and (v) in Fig. 1).

We can use high intensity ultrasound to pretreat target area and then apply dip and scrape MNs or super short MNs to deliver drugs. The inertial cavitation can reduce the resistance of SC layer so it will be easier for the MNs to create micro-abrasions on skin surface. This combination can provide a simple way to apply drugs that need rapid onset at topical area. The thermal effect comes along with the high intensity can also increase drug absorption rate (this combination can be referred to the categories (iii), (iv) and (v) in Fig. 1).

Although the MNs combined with sonophoresis methods show significant prospects, the consideration of MNs combined with other TDD technologies are also equally promising. Therefore, a cluster analysis has been employed in this work to reveal the current trends on these cooperating researches. The analysis is able to make suggestions on how these trends are likely to develop in the future.

The cluster analysis is a common statistical method used to discover useful information from different data groups (unlike time series analysis which uses the same time series data). The criteria of cluster analysis are flexible which means that it does not have a specific statistical algorithm [127]. In our case, four groups of subjects, namely, journal papers according to a specific area, are used for the analysis with 10 observations per subject counting from 2004 to 2013. The keywords used to acquire the data of the subjects (i.e., number of papers) are (i)

MNs, (ii) MNs and ultrasound and phono/sonophoresis, (iii) MNs and iontophoresis/electroporation, (iv) MNs and chemical enhancers.

We have used two specific concepts to design the clusters: the research activity and level of contribution from each research activity. The research activity is indicated by the number of publications of each year divided by the mean of the total number of publications of that subject group. The contribution level for each subject is defined as the number of publications of each year divided by the total number of publications of MNs of the same year. As the MNs papers cannot compare to themselves, the values of the contribution level of MNs are stochastically spread across the x axis. The four subjects are then plotted as four clusters in different shapes and colours (Fig. 15). The k-mean clustering method [128] is adopted to calculate the centroid of each cluster by measuring their squared Euclidean distance. The results of the cluster analysis (Fig. 15) indicate that both ultrasound and iontophoresis have shown relatively high research activities and levels of contribution. Specifically, the ultrasound combined with MNs shows the highest potential for research activity which suggests that this combination is developing fast in the past 10 years and more researches will be conducted in the future if the current trend continues. The iontophoresis and electroporation combined with MNs shows the highest contribution level which indicates that this is the research activity where the most current combinational researches are focused on.

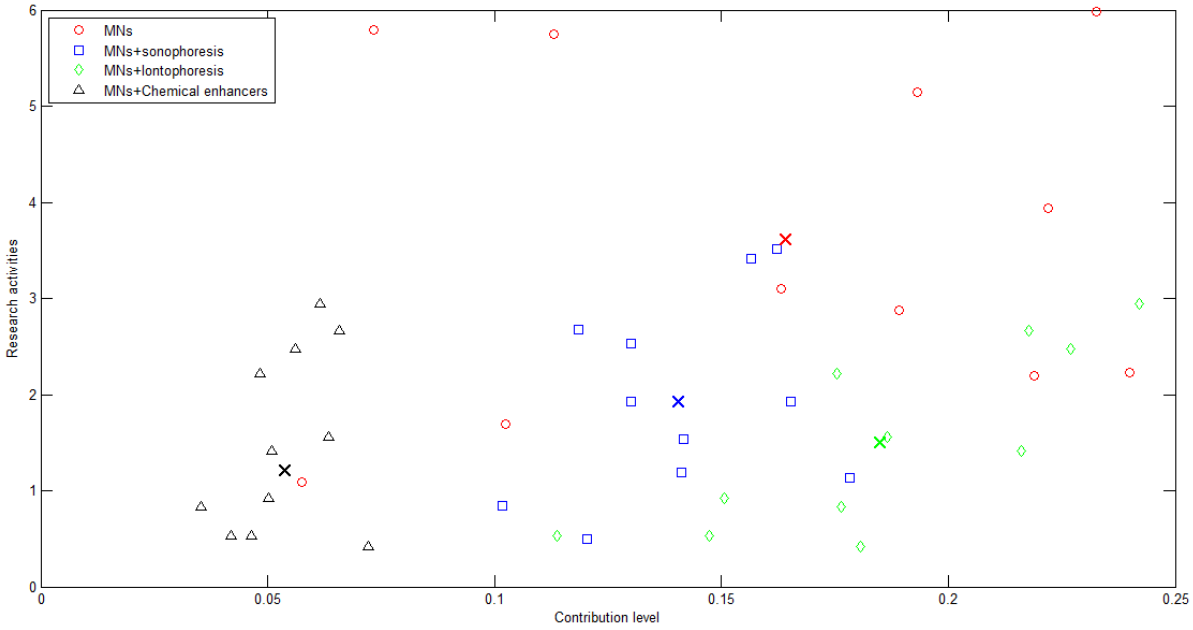

Fig. 15 Cluster analysis for determining the significance of current research on combined MNs with other TDD technologies

5. Conclusion

The TDD technologies are important drug delivery methods and they are developing fast as reviewed and forecasted in this paper. The idea of ultrasound and MNs combination is relatively new but shows a promising future. The forms and design of MNs patches will be more diverse in the future. Given that portable ultrasound instrument is a common medical device in hospitals, a patient friendly design of the combination are likely to be achieved at a reasonable cost when this concept is matured enough.

In order to discuss the scope of combined MNs and ultrasound research, this review paper has introduced the relevant mechanisms of MNs and sonophoresis as well as pointed out the weak areas in the researches of these two technologies. By working on those weak areas, more combinational approaches can be developed, particularly when the ultrasound and MNs are more developed as methods. Although the combination of MNs and ultrasound seems to

make TDD more complicated, it is a necessary step to achieve both higher and controllable drug delivery rate. In conclusion, we must state that the MNs and ultrasound combination has a promising future to solve some of the current problems in TDD.

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