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RESEARCH PAPER

Optimization of Impedance Spectroscopy Techniques for Measuring Cutaneous Micropore Formation after Microneedle Treatment in an Elderly Population

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ABSTRACT

Purpose The objective of this study was to optimize a reproducible impedance spectroscopy method in elderly subjects as a means to evaluate the effects of microneedles on aging skin.

Methods Human volunteers were treated with microneedles at six sites on the upper arm. Repeated impedance measurements were taken pre- and post-microneedle insertion. Two electrode types were evaluated (dry *vs.* gel), using either light or direct pressure to maintain contact between the electrode and skin surface. Transepidermal water loss (TEWL) was measured as a complementary technique.

Results Five control subjects and nine elderly subjects completed the study. Microneedle insertion produced a significant decrease in impedance from baseline in all subjects ($p < 0.05$, regardless of electrode type or pressure application), confirming micropore formation. This was supported by a complementary significant increase in TEWL ($p < 0.05$). The gel*direct condition produced the lowest variability between measurements, as demonstrated by a coefficient of variation of 3.8% and 3.5% (control and elderly subjects, respectively). This was lower than variation between TEWL measurements at the same sites: 19.8% and 21.6% (control and elderly subjects, respectively).

Conclusions Impedance spectroscopy reproducibly measures micropore formation in elderly subjects, which will be essential for future studies describing microneedle-assisted transdermal delivery in aging populations.

KEY WORDS elderly · impedance · microneedle · micropore · transdermal

ABBREVIATIONS

(CV%)	Coefficient of variation
(MN)	Microneedle
(SC)	Stratum corneum
(TEWL)	Transepidermal water loss

INTRODUCTION

Transdermal drug delivery provides significant advantages over other delivery routes, and these benefits can be afforded to skin-impermeable compounds through the use of microneedles (MNs). MNs are micron-scale projections that reversibly disrupt the stratum corneum (SC), the outermost skin layer that imparts most of the skin's barrier functions [1]. Insertion of MNs into the skin creates micropores (also known as microchannels or microconduits) in the SC, which significantly enhances skin permeability. These micropores allow percutaneous delivery of large compounds and hydrophilic substances into the systemic circulation, thus delivering compounds that are otherwise not able to cross the skin to any appreciable degree [2–4]. MN technologies would be ideal for

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elderly patients, allowing the benefits of transdermal delivery to be utilized with a greater number of drug therapies applicable to this population. In fact, transdermal drug delivery improves compliance with therapy in aging subjects [5], and the ease of administration and patch removal is highly beneficial. Aging skin has several differences from younger skin, including a thinner epidermis and reduced elasticity [6], and it is not yet known how these differences affect the skin response to MNs. The thinning epidermis was observed by Neerken *et al.*, in a study that compared elderly skin to a younger population [7]. They found the epidermis of the younger population to be 89±8 μm (forearm), where the epidermis of the elderly group was 75±7 μm (forearm).

In addition to preventing the ingress of xenobiotics, the SC also serves as the skin's barrier to the movement of ions and presents high impedance to the flow of electrical current. Concurrently, the skin also prevents excessive water loss from the body. These properties of the SC provide ideal means for monitoring skin barrier integrity. Measurement of impedance and water movement have both proven to be excellent techniques for assessing barrier function in healthy adults [8–12]. As such, the impedance spectrum decreases upon disruption of the SC, while transepidermal water loss (TEWL) increases in a complementary manner [9, 13–15]. Impedance techniques, however, are particularly useful in a clinical research environment. The ease of use is very high, the equipment is portable, and the method can detect small changes in the skin under a variety of conditions following MN insertion [11]. Impedance methods have been investigated for evaluating micropore formation following MN treatment in middle-aged subjects, and a variety of different electrode types and measurement techniques are available [11, 12]. Impedance techniques have yet to be specifically assessed in an elderly population. In order to fully explore the benefits of MN treatment in elderly subjects, a reliable measurement technique is essential for detecting micropore formation and predicting drug delivery. The objective of this study was to optimize an impedance spectroscopy technique in an elderly population as a means to evaluate the effects of MN treatment on aging skin.

MATERIALS AND METHODS

Clinical Study Procedures

All study procedures were approved by the University of Iowa Institutional Review Board and were completed according to the principles outlined in the Declaration of Helsinki. The study was conducted in the Clinical Research Unit at the University of Iowa Hospitals and Clinics. Healthy subjects between 18 and 95 years of age with no history of dermatologic disease were recruited. Exclusion criteria were similar to previous studies [12], including inability to give consent, severe allergies, previous adverse reactions to MN treatments, or the use of cholesterol lowering medications. Six sites were marked on the upper arm of each subject. Impedance and TEWL measurements were made at baseline (methods described below). Each site was cleaned with an alcohol wipe, followed by insertion of MN arrays. Impedance and TEWL measurements were then repeated post-MN insertion.

Microneedle Insertion

Each array consisted of 50 stainless steel MNs arranged in a 5×10 configuration, with each MN measuring 750 μm in length (Tech-Etch, Plymouth, MA; array design provided by the Prausnitz lab at the Georgia Institute of Technology). All arrays were autoclave sterilized before use, in order to closely mimic the conditions used when inserting a sterile hypodermic needle in clinical practice (the most closely related non-oral route of drug administration). The arrays were incorporated into patches using AR7717 adhesive backing (Adhesives Research, Glen Rock, PA) to provide secure contact of the MNs with the skin (thus allowing complete insertion of the MNs). The MNs were applied to the skin by gently pushing the array onto the skin for 15 to 20 s, followed by immediate removal. The microneedle array was then rotated and reinserted over the same site to create 100 total micropores (non-overlapping). All MN applications were performed by the same investigator to avoid variability in MN insertion.

Impedance Spectroscopy

Impedance measurements were made at baseline and following MN insertion at all sites. We used the same methods and study design that has been described previously [12, 16] in order to allow direct comparison of our results with prior studies. Two types of measurement electrodes were assessed. At three sites the measurements were made using Ag/AgCl electrodes with a "dry" gel measurement surface (T-3425 UniGel electrodes; Thought Technology Ltd., Montreal, Quebec, Canada). Measurements at the other three sites were made using electrodes with an Ag/AgCl wet gel foam electrode surface (Series 800 electrodes; S & W Healthcare Corporation, Brooksville, FL). A reference electrode (Superior Silver Electrode with PermaGel; Tyco Healthcare Uni-Patch, Wabasha, MN) was placed equidistant to the MN insertion sites. The electrodes (measurement and reference) were connected to an impedance meter (EIM-105 Prep-Check Electrode Impedance Meter; General Devices, Ridgefield, NJ) that applied a low-frequency alternating current modified with a 200 kΩ resistor in parallel (IET labs, Inc., Westbury, NY). Regardless of the electrode type (dry *vs.* gel),

six measurements were made at each site. The first three were made using light pressure to hold the electrode on the skin (using just the adhesive on the electrode to maintain contact with the skin). The other three measurements were made using more direct pressure, similar to the amount of pressure required to press an elevator button. Table I displays the study design.

TEWL Measurements

As a corresponding measurement to compare with the impedance, TEWL measurements were made at each site at baseline and immediately following MN insertion. Measurements were made using an open-chamber evaporimeter (cyberDERM Inc., Broomall, PA). The probe was lightly pressed to the subject's skin over each site until the reading become stable; TEWL units of $g{\cdot}m^{-2}{\cdot}h^{-1}$ were calculated by the equipment.

Data Analysis

With the current impedance spectroscopy setup, three electrical current pathways contribute to the total impedance measurement (Z_{total}): resistor box (Z_{box}), intact skin (Z_{skin}), and micropores (Z_{pores}). Because Z_{total}, Z_{box}, and Z_{skin} are known, Eq. 1 can be used to calculate the impedance of the micropores, as previously described [12, 16, 17]. This equation incorporates an assumption that the micropores comprise a total area of 2% under the electrode measurement surface.

$$Z_{total} = \frac{1}{\frac{1}{Z_{box}} + \frac{1}{Z_{skin}} + \frac{0.02}{Z_{pores}}} \quad (1)$$

Equation 1: Calculation of micropore impedance.

Additionally, the total permeable area ($A_{permeable}$) created from the 100 micropores at each measurement site was calculated according to Eq. 2 below [11]:

$$A_{permeable} = \frac{\rho L}{Z} \quad (2)$$

Equation 2: Calculation of the total permeable area formed by MN insertion [11, 12].

In this setting, ρ represents the electrical resistivity of interstitial fluid (~78 Ω-cm), L represents the estimated thickness of the outer skin layer (~15 μm), and Z represents the absolute impedance. Determining the total permeable area also allows the radii of each individual micropore to be calculated, using the assumption that each micropore contributes equally to the permeable area. In these studies, a 50 MN array was inserted twice to create

Table I Study Design and Description of Repeated Impedance Measurements at Six Sites on Subjects' Upper Arms

Baseline measurements (Six sites total per subject)			
Dry Ag/AgCl electrodes (n = 3 sites)		Gel Ag/AgCl electrodes (n = 3 sites)	
Light pressure (n = 3 per site)	n = 9 of each measurement type (across 3 sites)	Light pressure (n = 3 per site)	n = 9 of each measurement type (across 3 sites)
Direct pressure (n = 3 per site)		Direct pressure (n = 3 per site)	

↓

Microneedle insertion at each site

↓

Post-microneedle measurements			
Dry Ag/AgCl electrodes (n = 3 sites)		Gel Ag/AgCl electrodes (n = 3 sites)	
Light pressure (n = 3 per site)	n = 9 of each measurement type (across 3 sites)	Light pressure (n = 3 per site)	n = 9 of each measurement type (across 3 sites)
Direct pressure (n = 3 per site)		Direct pressure (n = 3 per site)	

100 non-overlapping micropores. Thus, it is assumed that each micropore will contribute 1/100th of the total permeable area.

For each electrode type-pressure combination, linear mixed model analysis was used to examine age group differences (elderly *vs.* control), and to confirm a significant change in the skin barrier function by comparing between baseline and post-MN values. The fixed effects in the model included age group, time (baseline *vs.* post-MN), and group*time interaction. Similar analysis was performed for TEWL. Linear mixed model analysis, with age group and pressure as fixed effects, was used for comparison of total permeable area and individual micropore radius between age groups and between light and direct pressures.

Variation between sites and between measurement replicates were assessed by fitting a random effects model for each age group, and electrode type and pressure combination. The variance component estimates from this fitted model were then used to compute the between site (and between replicate) standard deviation (SD) and coefficient of variation (CV%). The F-test was used to compare between site variance and between replicate variance for elderly *versus* control subjects. All statistical analyses were performed using SAS version 9.3. *P*-value <0.05 was considered statistically significant.

RESULTS

Subjects

Fourteen healthy volunteers completed the study. Five young adults (mean ± SD age of 24 ± 3.1 years) served as the control group, while the study group was composed of nine elderly adults (mean age of 73 ± 4.8 years). General demographics of the participants are described in Table II. MN treatment was well tolerated by all subjects, and no adverse reactions were noted at any of the treatment sites.

Impedance Measurements

Baseline to post-MN impedance measurements decreased significantly ($p<0.0001$) at all sites in both subject groups regardless of measurement technique or electrode types, indicating a breach in SC barrier function. The magnitude of change from baseline was generally greater in elderly subjects compared to controls for all four electrode-pressure conditions. The mean percent change from baseline for control *vs.* elderly for measurements made using dry electrodes with light pressure was −98.76% *vs.* −99.54% ($p=0.054$); for dry electrodes with direct pressure it was −99.68% *vs.* −97.88% ($p=0.0008$). For gel electrodes with light pressure it was −99.91% *vs.* −99.84% ($p<0.0001$), and for gel electrodes with direct pressure it was −99.78% *vs.* 99.65% ($p=0.001$).

Micropore impedance (Z_{pores}) was calculated at each site ($n=3$ measurements per technique) according to Eq. 1. The CV% between replications was calculated at each site for all impedance methods (data displayed in Table III). Variation between replicates was highest for both subject groups when measurements were made with dry electrodes, regardless of the pressure applied. In the control group, light and direct pressure techniques resulted in a CV% of 214.5% and 445.9% respectively, demonstrating extremely high variation between replicates. With the same electrode type in the elderly group, the CV% between replications was significantly lower compared to controls ($p<0.0001$) regardless of pressure applied during measurements, light (109.9%) and direct pressure (27.5%). Gel electrodes applied with light pressure produced significantly lower between replication CV% for the control group compared to the elderly group: 5.9% and 6.6% respectively ($p=0.018$). Gel electrodes applied with direct pressure did not produce significant differences between replications when comparing the subject groups ($p=0.245$). The between replication CV% was lowest when using the gel electrodes with direct pressure (young adults: 3.8%, elderly: 3.5%). Fig. 1 demonstrates the range of variability between replicates for all measurements made with gel electrodes.

Table II Subject Demographics

	Control group ($n=5$)	Elderly group ($n=9$)
Sex	2 males 3 females	5 males 4 females
Age (±SD), years	24 ± 3.1 (range: 20–27)	73 ± 4.8 (range: 65–78)
Race	2 Caucasian 3 mixed ethnicity	9 Caucasian

Transepidermal Water Loss

TEWL measurements significantly increased after MN treatment in both subject groups at all treatment sites ($p<0.0001$), complementing the impedance data and confirming micropore formation. The mean increase in TEWL from baseline ranged from 153.0 to 155.7% for control subjects, while a larger increase from baseline was observed in elderly subjects, ranging from 218.6 to 255.7% (Table IV). The magnitude of change from baseline was significantly higher for elderly subjects at the sites that were subsequently measured with gel electrodes ($p=0.002$); the data also suggested a possible difference between groups at the sites subsequently measured with dry electrodes ($p=0.057$). Because the between replication CV% was notably lower for impedance measurements made with gel electrodes (regardless of pressure applied), the CV% between replications and between sites was also calculated for the TEWL measurements. The between replication variation

Table III Between Replication Variation of Impedance Measurements Made on MN-Treated Skin

	Control group			Elderly group		
Electrode/Pressure	Between replication SD (95% CI)	Between replication CV%	Repeatability coefficient	Between replication SD (95% CI)	Between replication CV%	Repeatability coefficient
Dry electrode/Light pressure	137.5 (109.4, 184.9)	Mean = 64.1 214.5%	±381.0	34.3 (28.0, 44.1)	Mean = 31.2 109.9%	±95.0
Dry electrode/Direct pressure	299.0 (235.8, 408.6)	Mean = 67.0 445.9%	±828.7	1.6 (1.3, 2.1)	Mean = 5.9 27.5%	±4.5
Gel electrode/Light pressure	0.0098 (0.0078, 0.0131)	Mean = 0.167 5.9%	±0.027	0.0146 (0.0122, 0.0182)	Mean = 0.211 6.6%	±0.04
Gel electrode/Direct pressure	0.0063 (0.0020, 0.0084)	Mean = 0.165 3.8%	±0.017	0.0077 (0.0064, 0.0095)	Mean = 0.221 3.5%	±0.021

was not significantly different between the control and elderly groups: 14.7% and 22.8% respectively ($p = 0.1$). Variation between sites was 19.8% for control subjects, *vs.* 21.6% for the elderly subjects, which is higher than that observed from impedance measurements made with the gel electrodes at the same sites (Table V).

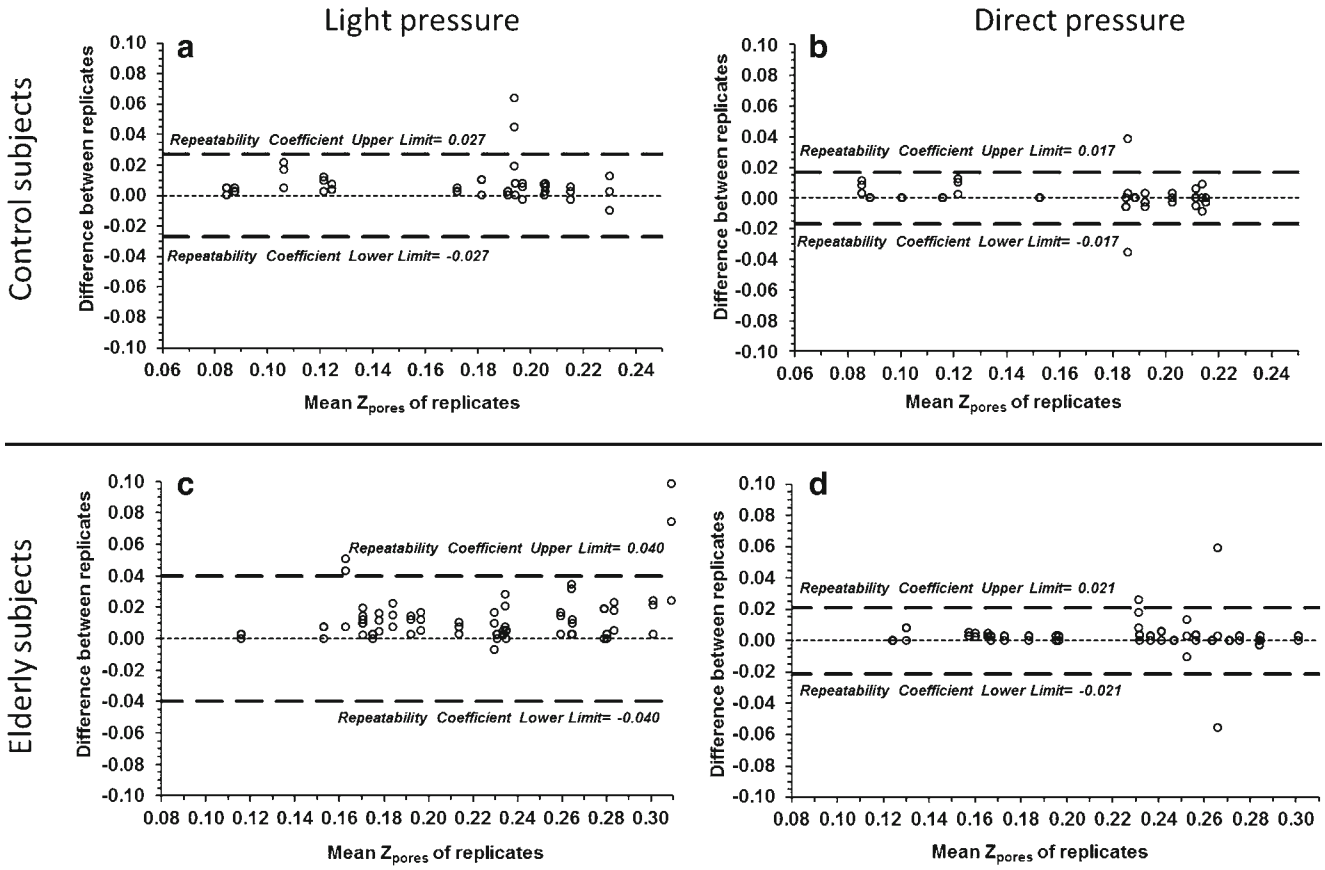

Fig. 1 Variation between replicates ($n = 3$ replicates per each treatment site) of impedance measurements made with gel electrodes on MN-treated skin (the lowest variability between measurements in our study was observed with gel electrodes applied with direct pressure on the MN-treated skin). The line at zero signifies no variability between measurements. The repeatability coefficient lines (a measure of precision) demonstrate the values in which an absolute difference between two impedance measurements would be expected to fall, with a 95% probability. Graph A: variation between replicates in control subjects ($n = 5$ subjects) when light pressure was applied during measurements; Graph B: variability between replicates in control subjects when direct pressure was applied (these are the same sites that were measured with light pressure, as displayed in Graph A). Graph C: variation between replicates in elderly subjects ($n = 9$ subjects) when light pressure was applied during measurements; Graph D: variability between replicates in elderly subjects when direct pressure was applied (these are the same sites that were measured with light pressure, as displayed in C).

Table IV TEWL Measurements Made at Baseline and After MN Treatment in Control and Elderly Groups

Sites	Time	Control group Mean ± SEM ($g\ m^{-2}\ h^{-1}$)	Elderly group Mean ± SEM ($g\ m^{-2}\ h^{-1}$)
1–3 (dry electrode measurement sites)	Baseline	6.65 ± 1.00	3.74 ± 0.44
	Post-MN	16.83 ± 2.52	11.92 ± 1.41
	% Change	153.0% 95% CI: (110.0%, 204.9%)	218.6% 95% CI: (174.9%, 269.2%)
4–6 (gel electrode measurement sites)	Baseline	6.15 ± 0.75	3.75 ± 0.36
	Post-MN	15.73 ± 1.93	13.34 ± 1.29
	% Change	155.7% 95% CI: (117.8%, 200.4%)	255.7% 95% CI: (213.2%, 303.9%)

Total Permeable Area and Individual Micropore Radius

The total permeable area created by MN treatment was calculated according to Eq. 2 for the sites at which gel electrodes were used for impedance measurements. When light pressure was applied, the mean (±SEM) $A_{permeable}$ was $11.61\times10^{-6}\pm2.14\times10^{-6}$ mm^2 for the control group and $11.44\times10^{-6}\pm1.20\times10^{-6}$ mm^2 for the elderly group. When direct pressure was used, $A_{permeable}$ was $17.97\times10^{-6}\pm2.38\times10^{-6}$ mm^2 for controls and $12.23\times10^{-6}\pm1.28\times10^{-6}$ mm^2 for the elderly group. There was no significant difference in $A_{permeable}$ between control and elderly subjects for either technique (light: $p=0.134$, direct: $p=0.087$).

The mean (±SEM) micropore radius in control subjects was 2.26±0.05 μm with light pressure measurements and 2.39±0.16 μm with direct pressure. The mean micropore radius in elderly subjects was 1.90±0.10 μm with light pressure and 1.97±0.10 μm with direct pressure (Fig. 2). There was no significant difference in mean radius between the control and older subjects, regardless of what pressure technique was used ($p=0.135$ and $p=0.089$ for light and direct pressure, respectively).

DISCUSSION

Over the next 15 years, the geriatric population (individuals ≥65 years of age) in the U.S. will grow steadily, with these individuals comprising the largest percentage of the total population [18]. As this subset of patients continues to increase in number, so will the associated healthcare costs. Chronic illness and degenerative conditions are more prevalent in patients over 65 years, and these patients are more likely to be taking a greater number of medications. In order to continually improve pharmacotherapy in aging populations, novel and alternative routes of drug administration need to be developed. However, this can be difficult due to the significant physiologic differences that occur with age and affect the body's response to treatment. The FDA recognizes that geriatric patients respond differently than young patients to drug therapy, with altered pharmacokinetics and pharmacodynamics, changes in enzyme systems, and increased sensitivity to adverse drug reactions. Drug delivery is also a significant challenge with pharmacotherapy in geriatric populations. These patients struggle to swallow solid dosage forms for oral use, experience difficulties with swallowing and palatability of liquid oral dosage forms (particularly when considering age-dependent taste sensation differences), and have difficulties with intravenous therapies. Transdermal drug delivery presents an attractive means for overcoming these challenges.

Transdermal delivery offers advantages that other forms of delivery cannot provide, many of which are especially pertinent for the geriatric population. Drug delivery through the skin avoids first-pass hepatic metabolism, eliminates the difficulties of swallowing oral medication, evades the need for venous access, and offers the possibility of rapidly terminating

Table V Between Site Variation for TEWL and Impedance Measurements Made with Gel Electrodes

Measurement	Control group		Elderly group	
	Between site SD (95% CI)	Between site CV%	Between site SD (95% CI)	Between site CV%
TEWL	3.21 (2.24, 5.63)	19.8%	2.98 (2.22, 4.53)	21.6%
Gel electrodes/light pressure	0.022 (0.015, 0.038)	13.1%	0.020 (0.015, 0.031)	9.1%
Gel electrodes/direct pressure	0.013 (0.009, 0.023)	8.1%	0.017 (0.012, 0.025)	7.5%

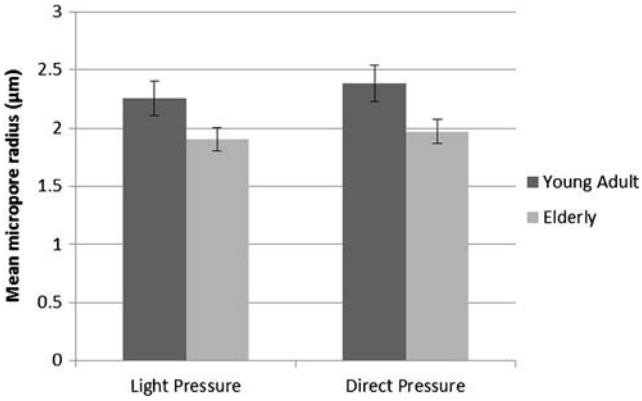

Fig. 2 Mean (±SEM) micropore radius following impedance measurements made with Ag/AgCl gel electrodes on MN-treated skin in young ($n = 5$) and elderly ($n = 9$) subjects. The micropore radii were calculated from the total permeable area created by the MN treatment (in this case, a total of 100 micropores were created and each micropore was considered 1/100th of the total area). There was no significant difference in micropore radius between young and older subjects ($p = 0.135$ for light pressure, $p = 0.089$ for direct pressure).

drug input. Additionally, transdermal administration achieves near zero-order delivery in which a drug is delivered at a constant and controlled rate [19]. This effect is particularly important for chronic therapies that would benefit from consistent drug plasma profiles (i.e. analgesics, cardiovascular agents, and hormones). Notably, many of these agents are typically prescribed for elderly patients [20]. Transdermal delivery significantly increases compliance with therapy in the elderly [5], but cutaneous delivery in general is an underutilized treatment option [18]. Despite the clear benefits of transdermal delivery for optimizing pharmacotherapy in elderly patients, the majority of medications do not possess the physicochemical properties required to passively traverse the skin. MNs provide a safe means for allowing percutaneous delivery of therapies that are not otherwise transdermal candidates.

MN Delivery in Elderly Populations

In clinical practice the advantages of MN techniques will be best applied to patients with unique drug delivery needs, including elderly patients. From a commercial perspective, the success of MNs is reliant on the ease of use; therefore, MNs ideally would be applied by the patients themselves, reducing dependence on a health care provider. Previous studies have shown that subjects are able to successfully self-administer MN treatment after receiving appropriate counseling of proper use, indicating that MNs are user-friendly [21, 22]. In this study, however, MN insertion was performed by members of the research team in order to reduce variability (which would have been introduced by self-application by the subjects). Given the methods development nature of this study, this was very important because of the potential for excess variability to complicate analysis of the measurement techniques.

While elderly patients are particularly well-suited for MN-assisted delivery, the intrinsic differences and unique needs of this population are often not considered in the development of new drug delivery techniques. MN treatment has been actively explored in middle-aged adults, but few studies have specifically described the effects of MN treatment in aging skin. Those studies that have investigated MN insertion in aging skin have focused on cosmetic applications (with no drug delivery) or drug delivery from a short patch wear time [23, 24]. Because of the notable differences in elderly skin compared to young skin (reduced epidermal thickness, decreased elasticity, and reduced microcirculation), it is clinically reasonable to assume that elderly skin may respond differently to MN insertion. For instance, it could be proposed that the difference in skin thickness might result in pain or pinprick bleeding with MN insertion in elderly subjects, as the MNs could reach and stimulate more of the dermal nerve endings and microvasculature. We did not observe either of these phenomena in our study, however. Additional differences in aging skin that could affect the response to MN insertion could include such components as differences in the level of barrier disruption achieved, or the timeframes by which the micropores permit drug delivery (especially with a longer patch wear time typical of many transdermal systems). Before any of these or other effects of MNs in elderly patients can be explored, however, it was first necessary to develop a technique capable of reliably measuring micropore formation in a clinical research environment.

Earlier studies have demonstrated that impedance spectroscopy is a clinically useful technique for measuring micropore formation in human subjects [11], focusing on parameters such as micropore viability and drug delivery timeframes [2, 17, 25]. These two factors are directly related, in that drug delivery ceases when the micropores are no longer viable. In this respect, impedance measurements can be viewed as a surrogate marker for predicting drug delivery time frames. This would prevent the need for invasive pharmacokinetic studies in the early stages of drug development, simplifying the drug screening process for a patient population that presents challenges with intravenous access (both for drug delivery and sample collection). In order to use impedance techniques in elderly patients, however, it was critical to ensure that the method can reliably detect micron-scale changes in the skin without introducing excessive experimental noise.

Minimizing Experimental Variability

A variety of electrode types and measurement techniques can be used to make impedance readings on the skin. We compared two electrode types that have different surface electrode properties: one with a "dry" gel measurement surface and the

other with a wet gel surface. This comparison has been made previously in young adults, and the gel electrodes were shown to be superior for reducing variability between measurements [12]. Our results demonstrate the same trend for elderly subjects. Both electrode types and pressure techniques were able to detect a significant breach in the skin after MN insertion (which was complemented by the TEWL data). However, the variability between replicates was notably different between the electrode types. Repeated measurements with dry electrodes (regardless of the pressure applied) yielded unacceptable variability for a clinical measurement technique, as demonstrated by a CV% ranging from 27.5 to 109.9% in the elderly subjects. The variabilty was even higher in the younger subjects (Table III). In the context of trying to measure micropore formation and viability, this level of variability would render the technique ineffective for distinguishing changes in the micropores *vs.* other confounding factors (inter-individual variability, skin hydration, etc.). Conversely, the gel electrodes notably minimized variability between measurements. As a result, the between replicate CV% was reduced to a range of 3.5–6.6%, which is exceptional for clinical data. Additionally, variation between treatment sites was very low and nearly identical between populations (8.0% and 7.9% for control and elderly subjects, respectively). This demonstrates that impedance measurements, particularly when using gel electrodes with direct pressure, can precisely measure micropore formation regardless of treatment site or subject age. The variation between replicates and between sites was even lower than what was observed with TEWL (22.8% and 21.6%, repectively). This is especially encouraging given that TEWL measurements require software for data collection and are more sensitive to changes in skin hydration status. For this reason, impedance measurements are somewhat more amenable to a clinical research envivonment, and now we have techniques that can garner more precise data for measuring micropore formation.

Micropore Area Available for Drug Diffusion

From a practical perspective, the amount of drug permeation is directly correlated with the area available for diffusion (in this case, described by the calculated total permeable area). This provides a numerical target to correlate with steady-state drug flux (determined from *in vitro* and/or *in vivo* drug diffusion studies). Additionally, this calculation can incorporate the effect of factors that can be experimentally varied (MN geometry, number of MNs, occlusion of the site, etc.). In this case, the $A_{permeable}$ was not significantly different between control and elderly groups, suggesting that there will not be a difference in the amount of drug diffusion *in vivo* based on the age of the subject. It can also be assumed that dosing adjustments will not be necessary when treating elderly patients with MN arrays. An additional application of the $A_{permeable}$ calculation is to numerically describe how the pathways for drug diffusion progressively decrease in size as the skin restores its barrier function. This will be particularly important for future studies as the micropore healing times could be different based on subject age, which would affect the timeframe by which the micropores permit drug diffusion. Previous studies have demonstrated that wound healing in elderly patients is not qualitatively altered, but tends to be delayed when compared to middle-aged patients [26].

Limitations

Some limitations exist in this work. First, this was a small study with a low number of participants. However, making multiple measurements at each treatment site and comparing baseline to post-MN treatment within each subject allowed us to determine significant trends without the expense of a larger study. Additionally, the values for ρ (electrical resistivity of interstitial fluid in the skin) and L (SC thickness) that were used to calculate the total permeable area of the micropores were based on values for middle-aged adults [11, 12]. Thus, our calculations employ the assumption that these properties are similar between younger and elderly adults. Given that epidermal thickness decreases with age, it could be possible that the SC would be thinner in elderly adults. In this case, our calculations of $A_{permeable}$ would be overestimated for the elderly population. On the other hand, it is known that the SC can become thicker as a result of chronic UV radiation, which could also be a possibility in elderly subjects. In that case, our calculations would be underestimated. However, drug permeability through the skin is affected by the interaction of a number of factors beyond just the formation of micropores. These factors include such components as skin hydration, drug concentration and formulation, and physicochemical properties of the compound. Therefore, a slightly over or underestimated $A_{permeable}$ is not likely to be a clinically significant factor that will greatly affect drug delivery. Our data would have been strengthened by including these factors and correlating them with the impedance measurements, but that work was beyond the scope of this small methods development study.

While impedance and TEWL are suitable methods to measure skin disruption due to MN application, they do not provide information about the depth or extent of penetration into the tissue. Recent studies have shown that optical coherence tomography (OCT) can visualize inserted MN arrays, which allows for penetration depth to be determined [27, 28]. OCT has the potential to replace other methods for determining MN penetration depth, which includes histological sectioning and staining that may damage the skin samples. While determining penetration depth is important to consider during MN use (particularly in drug delivery settings), it was not directly necessary for developing an impedance measurement

technique, which would certainly be considered a surrogate measurement. For this reason, including a method such as OCT for measuring the depth of MN penetration was not included in this study, but would be relevant for future studies of MN-assisted drug delivery and skin recovery times in elderly subjects. Last, we asked each subject about tolerability of the MN insertion process, but we did not formally evaluate pain using a VAS or other pain scoring method.

CONCLUSIONS

This is the first work to specifically develop an impedance technique for measuring micropore formation in an elderly population. Our future studies will employ this method to characterize the response of elderly skin to the effects of MNs in terms of drug delivery, healing times, and irritation potential. Overall this technique will contribute to a more comprehensive understanding of how MN treatment can assist transdermal delivery in aging adults.

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