Journal of Controlled Release 225 (2016) 294–300

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Micropore closure kinetics are delayed following microneedle insertion in elderly subjects

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ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form 1 December 2015
Accepted 27 January 2016
Available online 30 January 2016

Keywords:
Microneedle
Transdermal
Elderly
Drug delivery
Micropore
Impedance

ABSTRACT

Transdermal delivery is an advantageous method of drug administration, particularly for an elderly population. Microneedles (MNs) allow transdermal delivery of otherwise skin-impermeable drugs by creating transient micropores that bypass the barrier function of the skin. The response of aging skin to MNs has not been explored, and we report for the first time that micropore closure is delayed in elderly subjects in a manner that is dependent upon MN length, number, and occlusion of the micropores. Twelve control subjects (25.6 ± 2.8 years) and 16 elderly subjects (77.3 ± 6.8 years) completed the study. Subjects were treated with MNs of 500 μm or 750 μm length, in arrays containing 10 or 50 MNs. Impedance measurements made at baseline, post-MN insertion, and at predetermined time points demonstrated that restoration of the skin barrier is significantly slower in elderly subjects under both occluded and non-occluded conditions. This was confirmed via calculation of the total permeable area created by the micropores (which would approximate the area available for drug delivery), as well as calculation of the micropore half-life. This pilot study demonstrates that longer timeframes are required to restore the barrier function of aged skin following MN insertion, suggesting that drug delivery windows could be longer following one treatment with a MN array.

1. Introduction

Transdermal drug delivery via patches that adhere to the skin offers many therapeutic advantages that are particularly appropriate for elderly patients. Drugs can be passively delivered in a zero-order fashion (constant delivery over time), thereby bypassing first-pass metabolism, erratic bioavailability, and the pain of parenteral injections. Therapy can be rapidly terminated as needed via patch removal, and application of additional patches can augment therapy when appropriate.

This route of drug delivery is generally limited to a small number of compounds due to the outermost layer of the skin, the stratum corneum, which provides the barrier function of the skin [1]. Microneedles (MNs) are a well-tolerated, minimally invasive means of overcoming the SC with the primary goal of broadening the number of drugs available for transdermal delivery. MNs temporarily disrupt the stratum corneum through the formation of aqueous channels, or micropores, that allow a topically applied drug compound to reach the systemic circulation by way of the dermal microcirculation [2,3]. MN-assisted delivery would present significant advantages for the elderly by offering the ability to administer a greater number of drug compounds transdermally. This is particularly important due to the high frequency of chronic medical conditions and complicated medication regimens in this population. However, the intrinsic differences and unique needs of this population are often not considered in the development of new drug delivery techniques. A clear understanding of how aged skin responds to MN insertion will allow safe and effective transdermal delivery systems to be developed for common conditions in the elderly.

The skin undergoes structural and functional changes with age [4–6]. In particular, aged skin has less elasticity, a flattened dermal-epidermal junction, and reduced hydration when compared to younger skin [7,8]. Additionally, decreased stratum corneum hydration and slower wound healing occur in aged skin [9,10]. It is not known how these differences affect the skin's ability to restore barrier function following MN insertion, as micropore closure kinetics have not been studied in elderly subjects. The amount of barrier disruption and the rate of micropore closure in young, healthy adults correlate with MN geometry and occlusion of the MN-treated skin [11,12]. It is important to study these parameters in elderly subjects as well, to determine the effect of aging on the kinetics of micropore closure. The rate of micropore closure

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http://dx.doi.org/10.1016/j.jconrel.2016.01.051
0168-3659/

is particularly important because this directly correlates to the timeframe by which a drug can be delivered transdermally.

Age-dependent differences in rates of micropore closure will directly affect the duration and extent of systemic drug delivery. Delineation of these parameters would permit tailored delivery of medications, which will promote healthy aging. Thus, for reasons of safety and efficacy it is necessary to understand how the closure of micropores differs in aged vs. young populations. Here we present the first studies to examine formation and closure of micropores in elderly human subjects. Our data show for the first time that micropore resealing is delayed in the elderly, and the closure rates are dependent upon MN number, length, and occlusion of the skin.

2. Methods and materials

2.1. Microneedle arrays and occlusive coverings

Four MN geometries were evaluated (Table 1). Stainless steel MN arrays consisted of either 10 MNs (arranged in a 2×5 configuration) or 50 MNs (arranged in a 5×10 configuration), with MNs measuring either 500 μm or 750 μm in length (Tech-Etch, Plymouth, MA). Occlusive patches were fabricated by attaching a rubber ring to a drug impermeable backing membrane (Scotchpak 1109 SPAK 1.34 MIL heat-sealable polyester film; 3M, St. Paul, MN) using 3M double-sided medical tape. The patches were held to the skin with a transparent film dressing (Tegaderm™; 3M, St. Paul, MN). MN arrays and occlusive patches were autoclave sterilized before use.

2.2. Clinical study procedures

All study procedures were approved by the University of Iowa Institutional Review Board and followed guidelines set forth by the Declaration of Helsinki. The study took place in the Clinical Research Unit at the University of Iowa Hospitals and Clinics. Written informed consent was obtained from subjects prior to being enrolled in the study. Healthy volunteers between 18 and 30 years of age (control group) and ≥65 years of age (elderly group) were recruited for the study. Exclusion criteria included inability to give consent; previous adverse reaction to MN treatment; known allergy to latex, rubber, or other adhesive tape; or the use of the following medications: HMGCoA reductase inhibitors ("statins"), steroids, or antibiotics. Individuals who were pregnant/nursing or diagnosed with diabetes or HIV/AIDS were also excluded from the study.

Subjects were randomly assigned to one of four treatment groups (Table 2). Subjects in Groups 1 and 1A were treated using MN geometries A and B; subjects in Groups 2 and 2A were treated using MN geometries C and D. MN-treated sites for subjects in Groups 1A and 2A were covered with occlusive patches.

2.3. Experimental procedures

Impedance spectroscopy methods were similar to those described in previous studies [11,13,14]. Ag/AgCl wet gel foam electrodes (Series 800 electrodes; S&W Healthcare Corporation, Brooksville, FL) were used to obtain measurements at each of the MN-treated sites. A reference electrode (Superior Silver Electrode with PermaGel; Tyco Healthcare Uni-Patch, Wabasha, MN) was placed equidistant to the MN-treated sites. The measurement and reference electrodes were connected by lead wires to an impedance meter (EIM-105 Prep-Check Electrode Impedance Meter; General Devices, Ridgefield, NJ) which applied a low frequency, alternating current modified with a 200 kΩ resistor in parallel (IET labs, Inc., Westbury, NY). Impedance measurements were made in triplicate at each time point.

Six sites on the upper arm were marked with a pen and baseline impedance measurements were made at each site. Two additional sites on the opposite arm were included for subjects in the occluded condition groups (Groups 1A and 2A). These sites did not receive MN treatment and served as control sites to estimate the confounding effect of hydration on the measurements. Following baseline measurements, isopropyl alcohol pads were used to cleanse each of the six sites. Three sites were treated with a MN array of one geometry, and the remaining three sites were treated with another geometry of the same length but different number of MNs (Table 2). Immediately before insertion, the MN arrays were made into patches using AR7717 adhesive backing (Adhesives Research, Glen Rock, PA) in order to provide secure contact with the skin and allow complete insertion of the MNs. The MNs were applied to the skin by gently pressing the MN array perpendicular to the skin surface for 15 to 20 s, after which the array was removed. At sites treated with arrays containing 50 MNs, the array was rotated approximately 45° and reinserted over the site to create 100 non-overlapping micropores. Impedance measurements were taken immediately following MN insertion at all sites.

In Groups 1 and 2 (non-occluded conditions), impedance measurements were taken every 30 min up to 4 h post-MN. Subjects returned to the Clinical Research Unit the following day for a final impedance measurement at 24 h post-MN treatment. In Groups 1A and 2A (occluded conditions), occlusive patches were used to cover the control and MN-treated sites. Subjects returned to the Clinical Research Unit at 24, 48, 72, and 96 h post-MN treatment. At each visit, impedance measurements were taken and new occlusive patches were applied. At the time of each impedance measurement the MN-treated sites were visually inspected for signs of irritation (i.e., redness, warmth, edema).

Table 1
Parameters of the four microneedle geometries studied. The arrays of 50 microneedles were inserted twice to create a total of 100 non-overlapping micropores.

Geometry	Microneedle length (μm)	Number of microneedles	Total number of micropores created
A	500	10	10
B	500	50	100
C	750	10	10
D	750	50	100

Table 2
Microneedle geometries and occlusion conditions assigned to the four treatment groups.

Group	Geometry	Occluded
1	A and B	No
1 A	A and B	Yes
2	C and D	No
2 A	C and D	Yes

2.4. Data analysis

2.4.1. Calculation of micropore impedance, total permeable area and individual micropore radius

The mean impedance value was calculated for each site at each time point. Individual measurements were excluded from analysis if they were ≥125% different than the other two measurements at that site (approximately 15 kΩ different from the other two measurements, which is similar to previous work [13]). Additionally, impedance values were excluded if there was no significant difference between pre- and post-MN measurements (indicating the lack of a breach in barrier function).

With this impedance setup there are three parallel and independent pathways: resistor box (Z_{box}), intact skin (Z_{skin}, pre-MN baseline), and micropores (Z_{pores}). The values for Z_{total}, Z_{box}, and Z_{skin} are known, permitting the impedance of the micropores to be calculated utilizing Eq. (1) [13]:

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