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In vitro characterization of the invasiveness of polymer microneedle against skin

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ABSTRACT

The micro-sized needles could pierce the skin to deliver drugs effectively in a minimally invasive and painless manner. However, there are only a few reports that identify the invasiveness and painlessness of microneedle (MN), and in vitro characterization studies were conducted to examine the invasiveness of MN in experimental animals and healthy volunteers. First, a fluorescent molecule was applied to show the skin holes according to the application time of MN and then the whitening effect in UV-exposed hairless rats was observed using reflectance spectroscopy according to the application time of MN. The extent of skin irritation by the application time of MN in healthy volunteers was determined from the value of skin redness. Regardless of MN application time, skin redness occurred and then disappeared 30 min after removal of MN; this phenomenon was insignificant with the application time of MN. Thus, if the MN was applied, a skin hole appeared, skin redness was observed and then the skin redness disappeared 30 min after removal of MN. Taken together, polymer MN might be a suitable tool for safe transdermal drug delivery of small molecules.

1. Introduction

Transdermal drug delivery offers many important advantages. For example, it is easy and painless, it protects the active compound from gastric enzymes, and it avoids the hepatic first-pass effect. However, the skin is a natural barrier and only a few drugs can penetrate the skin easily with the aid of penetration enhancers such as soybean phospholipids (Kirjavainen et al., 1999), long-chain fatty alcohols, cyclic monoterpenes (Parsaee et al., 2002; Ho et al., 1994) and non-ionic surfactants (Shin et al., 2005). However, the stratum corneum (SC) limits these methods to the delivery of potent drugs that are hydrophobic and low molecular weight. Biopharmaceuticals, such as peptides, proteins and the future use of DNA and RNA, are a rapidly growing segment of pharmaceutical therapies (Wilke et al., 2005; Martanto et al., 2006). These biotechnology drugs are currently delivered almost exclusively through the parenteral route, which creates some problems in delivery, such as accidental needle sticks or pain resulting in reduced patient compliance. Therefore, a new concept, a microneedle (MN) array, was introduced to merge the advantages of transdermal delivery and parenteral delivery (Verbaan et al., 2008; Aoyagi et al., 2006).

Recently, MN of silicon (Kim et al., 2006), glass (Ayittey et al., 2009), metal (Li et al., 2010), or polymers (Han et al., 2007) have been proposed to deliver macromolecules, like BSA (Xie et al., 2005) and insulin (Chen et al., 2009), and small molecules, like ketoprofen (So et al., 2009), calcein and vitamin C (Oh et al., 2008; You et al., 2010). However, few studies of the safety issues of MN have been conducted. Bal et al. (2008) published the safety in terms of skin irritation (skin redness and blood flow) and pain sensation from stainless steel MN, and for all MN, the irritation was minimal and lasted less than 2 h. In this study, the authors tried to identify the invasiveness of polymer MN by measuring the skin irritation according to the application time of polymer MN.

2. Materials and methods

2.1. Volunteers

Five healthy volunteers (men only), aged between 22 and 27 years and with no pre-existing skin conditions, participated in the study after giving their informed consent. They were asked not to apply any cosmetic formulations on the left arm before the studies.

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2.2. Materials

HPLC-grade methanol was obtained from JT Baker Inc. (Phillipsburg, NJ). Vitamin C and fluorescein isothiocyanate (FITC) were purchased from Sigma (Steinheim, Switzerland). Seven-week-old hairless rats were purchased from SLC Inc. (Shizuoka, Japan). Carbomer 940 was used as received without further purification. All other chemicals and solvents were of analytical reagent grade and used without further purification.

2.3. Fabrication of MN

The biocompatible MN was fabricated by Han et al. (2007). Briefly, the in-plane MN was fabricated using inclined UV lithography and electroforming with a sharp tip for a low insertion force and was made long enough to ensure sufficient penetration depth. In this step, it was easy to control the length, side shape, and tip sharpness. The in-plane MN was then converted into an out-of-plane MN array in order to increase the needle density. For mass production, a negative mold was fabricated by replicating the out-of-plane MN array. Finally, the out-of-plane MN sheets were produced from polycarbonate for biocompatibility using the negative mold in a hot embossing machine. The height of the fabricated PC MN was 500 μm and the density was 154 ea/cm^2.

2.4. Vitamin C gel

First, exactly 1 g of vitamin C was dissolved in 10 mL of ethanol and moderately stirred for 10 min. At the same time, 1 g of carbomer 940 was completely hydrated in 80 mL of distilled water and then vitamin C solution was added to the carbomer 940 solution. The mixtures were stirred using a magnetic stirrer, and the vitamin C gel was formed with triethanolamine.

2.5. Skin hydration by electrical conductance measurement

Electrical conductance measurements were taken with skin surface hygrometer (Skicon-200EX, IBS CO., Japan) to check the effect of MN on skin hydration. Male hairless rats, HWY/slc, with a body weight of 250 ± 30 g were purchased from SLC Inc. (Shizuoka, Japan). All animals had free access to food and water and were kept in an air-conditioned room under a 12-h dark/light cycle. During the experimental period, the hairless rats received care consistent with the guidelines of Chungnam National University. The back of each hairless rat was divided into three sections and electrical conductance was measured before application of the polymer MN. At pre-determined intervals after application of the polymer MN for 2, 10, 30, 60 and 240 min, electrical conductance was measured.

2.6. Skin whitening applied by MN into hairless rats

The skin whitening effect of vitamin C in the presence of MN against UV-induced pigmentation was investigated in hairless rats (Kobayash, 2006). Male hairless rats, HWY/slc, with a body weight of 250 ± 30 g were purchased from SLC Inc. (Shizuoka, Japan). All animals had free access to food and water and were kept in an air-conditioned room under a 12-h dark/light cycle. During the experimental period, the hairless rats received care consistent with guidelines of Chungnam National University. The back of each hairless rat was divided into four sections with an area of 2 cm^2 and irradiated by UV lamp (Samsung US-840MA, Korea) for 7 days. The hairless rat's back was then treated twice a day for 4 days as follows: group 1 as the control received 0.1 g/rat of vitamin C gel only, group 2 received MN application for 10 min after 0.1 g/rat of vitamin C gel, group 3 received 0.1 g/rat of vitamin C gel after application of MN for 10 min, group 4 received 0.1 g/rat of vitamin C gel after application of MN for 60 min. The skin redness was measured after 2, 4, 6, 10, 14 and 18 h by a reflectance spectrophotometer (Konica Minolta, Japan).

2.7. Confocal images of skin after MN application

The ability of MN to create transport pathways across the SC was assessed using FITC according to the elapsed time of MN application onto the hairless rat skin. Five μg/mL of FITC was loaded on the hairless rat skin for 360 min as a control and MN were applied as follows: 5 μg/mL of FITC coupled with MN for 6 h, 5 μg/mL of FITC coupled with MN for 1 h, and 5 μg/mL of FITC coupled with MN for 10 min. Rat skin sites treated by MN were subsequently removed and frozen, and the fluorescence of the rat skin was observed using confocal microscopy (LSM5 live configuration Variotwo VRGB, Zeiss, USA).

2.8. Skin irritation following MN in human skin

The skin irritation by using MN having the geometry of 500-μm depth and 154 ea/cm^2 was examined for clinical use of MN, eventually. MN was applied on the left arm of five healthy volunteers for 2, 10, 30, 60, and 240 min. The value of skin redness was measured with a reflectance spectrophotometer according to the elapsed time after removal of MN.

2.9. Statistical analysis

The Student's *t*-test was used to compare two different groups of samples. A p-value <0.05 was considered significant.

3. Results and discussion

3.1. Skin hydration by electrical conductance measurement

The water content of SC is one of the key factors regulating skin health (Rawlings and Matts, 2005). It affects the permeability and flexibility of SC and modulates the activities of several enzymes involved in the processes of barrier formation and desquamation (Leyden and Rawlings, 2002; Mak et al., 1991). SC hydration exhibits a steep gradient from the skin surface to the viable epidermis (Warner et al., 1988). Knowledge of the water content and distribution throughout the SC is thus highly relevant for numerous medical (i.e., physiological health), cosmetic (i.e., perceived appearance) and pharmaceutical (i.e., transdermal drug administration) applications (Rawlings, 2003; Rawlings and Harding, 2004; Loden, 2003; Naik et al., 1999). The ability of an alternating current to flow through the SC is an indirect measure of its water content (Berardesca et al., 1997). After application of polymer MN, the values of electrical conductance were recorded, which represented the water amount due to the occurrence of skin holes. The electrical conductance at different time points after the application of polymer MN was compared to the electrical conductance before application. The polymer MN was applied at t = 0 min. When the polymer MN was applied to rat skin, the values of electrical conductance were sharply increased in a manner dependent on the application time (Fig. 1). Those values were steeply decreased at 10 min, then gradually decreased at 30 min after removal of polymer MN, and reached the baseline observed prior to application of polymer MN at 420 min after removal of polymer MN. These results were consistent with findings that the conductance of tape-stripped SC correlated with the water content under appropriate conditions (Boncheva et al., 2009).

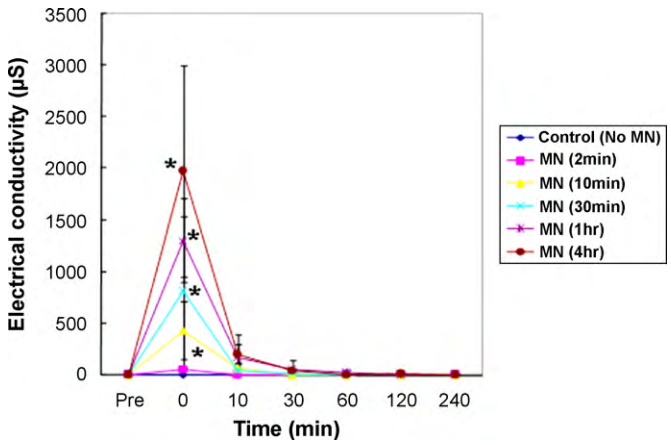

Fig. 1. The changes in electrical conductance at different time points after the application of polymer MN.

3.2. Confocal images of skin after applying MN

The insertion effect in the skin by polymer MN having various array densities (154 ea/cm^2, 99 ea/cm^2 and 45 ea/cm^2) under the same pressure for 30 min were previously published in assessments of the ability of polymer MN to create transport pathways across the SC, and the results showed that there was an increasing number of holes on the dermal side of the skin with increasing density of polymer MN (Oh et al., 2008). However, we had not reported the depth of the transport pathway across the SC according to the application of polymer MN. Therefore, we checked whether the depth of the skin holes elicited by MN increased as the application time of MN increased. When applying FITC solution only, FITC could not penetrate into the skin, even after 360 min, but rather stayed at the SC, indicating that SC was a physical barrier to FITC. However, the depth of skin holes gradually increased by more than 200 μm when FITC was loaded after the application of polymer MN for 10 min, 1 h and 6 h (Fig. 2). According to this result, as the application time for MN gets longer, the depth of the holes gets deeper. Furthermore, the space between the holes and the depth of holes were matched to the MN array. Also, the end of the polymer MN tip was intact (Fig. 3), suggesting that polymer MN had a strong integrity. Once skin holes were created according to the application time of the polymer MN and the holes were very solid, they were visualized by optical microscopy and confocal microscopy. However, visualization to show the recovery of skin holes was impossible because the holes gradually disappeared after removal of polymer MN and the skin holes could not be observed. Therefore, skin irritation studies measuring the skin color by reflectance spectrophotometry were conducted.

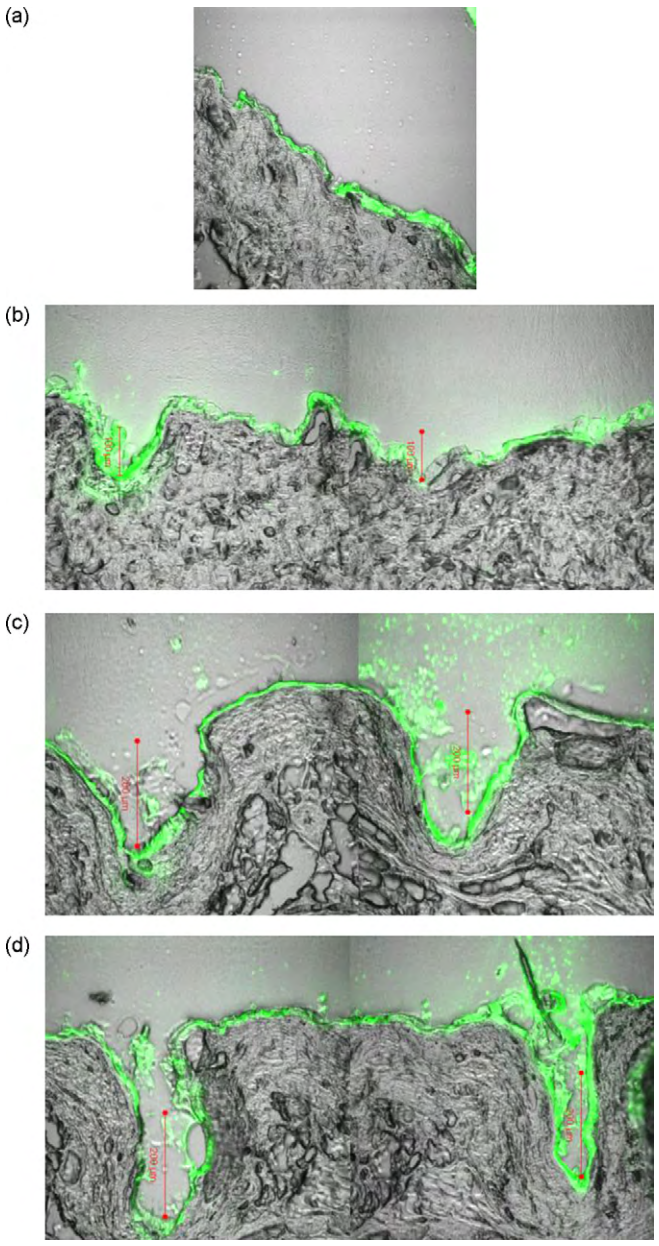

Fig. 2. The confocal microscope images of rat skin section by application of FITC coupled with polymer MN. (a) FITC applied to the intact skin; (b) FITC coupled with MN application for 10 min; (c) FITC coupled with MN application for 1 h; (d) FITC coupled with MN application for 6 h.

3.3. Skin irritation followed by MN in human skin

Erythema, one of the fundamental markers of inflammation, can be measured by both chromameter and laser Doppler imaging (LDI) methods (Corsini and Galli, 2000). However, a chromameter measures only the superficial redness, while LDI measures the blood flow much deeper in the skin (Larsson et al., 2002). Based on the results of FITC imaging according to the application of polymer MN, namely that the skin holes disappeared after removal of the

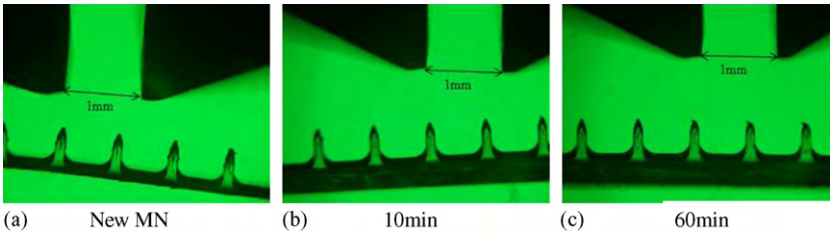

Fig. 3. The integrity of MN according to application time on the skin. (a) New MN; (b) MN after insertion for 10 min; (c) MN after insertion for 60 min.

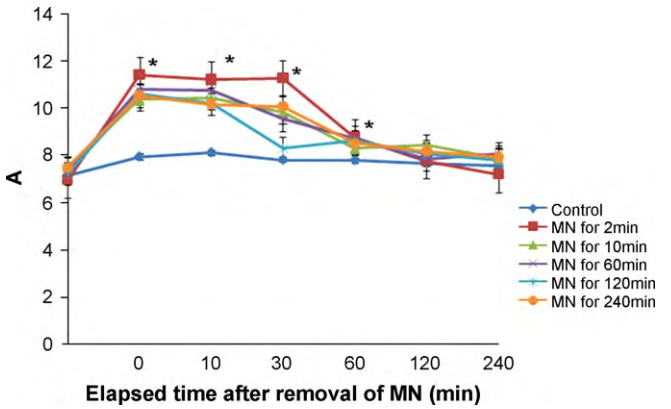

Fig. 4. Skin irritation by reflectance spectrophotometer according to MN application time and elapsed time after removal of MN.

polymer MN, the chromameter method was used to assess skin irritation. The skin redness was examined as a determinant of the degree of irritation. Fig. 4 shows the change in redness for the polymer MN according to the application time. The redness at different time points after the application of polymer MN were compared to the redness before application. The polymer MN was applied at $t = 0$ min. The redness score was increased to 10.376–11.44 absorption units right after application of polymer MN. The highest initial value of redness was measured when MN was applied for 2 min and was maintained for 30 min, indicating that redness was greater when the application time was shorter, explaining the recovery of skin barrier function (Zhou et al., 2010). There was little difference in the decrease of redness after MN application based on application time, but redness was generally maintained until 30 min and rapidly decreased between 30 min and 2 h in Fig. 4.

3.4. Skin whitening by MN application in hairless rats

After the hairless rats were irradiated for 7 days with a UV lamp, the value of skin redness increased to 3.1–4.45 compared to 1.25–2.06 before irradiation. The mitigating effect of the polymer MN on UV-induced skin redness was investigated. When MN was applied for 10 min after treatment with 0.1 g of vitamin C gel to rat, the value of skin redness was reduced by 2.26-fold 10 min after the removal of polymer MN compared to that after application of vitamin C gel only (Fig. 5). Thus, when vitamin C gel was applied together with polymer MN to the rat, faster recovery than normal against skin redness could be expected.

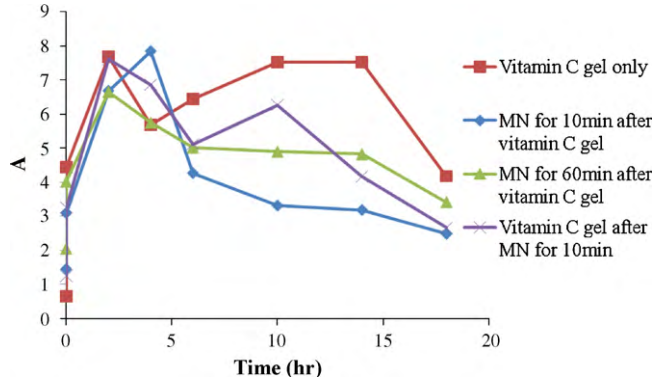

Fig. 5. The mitigating effect on UV-induced skin redness.

4. Conclusion

The aim of this study was to investigate the ability of MN to disrupt the barrier of the skin and to determine the safety of MN treatment in terms of skin irritation as measured by reflectance spectrophotometer. When the polymer MN was applied to rat skin, the values of electrical conductance were sharply increased in a manner dependent on the application time. At 420 min after removal of polymer MN, those values eventually reached the baseline observed before application of polymer MN. These results correlated with the skin redness after application of polymer MN, which increased and then recovered to baseline. As the application time of MN was increased, the length of the hole got deeper. This indicated that MN application time was an important point for barrier disruption. There was little difference in the decrease of redness after applying MN for various times, but redness was generally maintained until 30 min and then rapidly decreased between 30 min and 2 h.

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