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Note

Mechanism of fluid infusion during microneedle insertion and retraction

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Abstract

Previous work has shown that infusion flow rates can be increased by an order of magnitude by partially retracting microneedles after insertion into the skin. This study sought to determine the mechanism by which retraction increases fluid infusion by piercing human cadaver skin with single microneedles, fixing the skin after retracting microneedles to different distances, and examining skin microstructure by histology. We found that microneedle insertion to 1080 μm from the skin surface resulted primarily in skin indentation and only 100–300 μm penetration into the skin. This caused significant compaction of the skin, which probably pressed out most water and thereby dramatically lowered the flow conductivity of skin beneath the needle tip. Retraction of the microneedle allowed the skin to recoil back toward its original position, which relieved the skin compaction and increased local flow conductivity. Altogether, these results suggest that microneedle insertion to penetrate into the skin followed by microneedle retraction to relieve skin compaction is an effective approach to infuse fluid into the skin in a minimally invasive manner.

Keywords: Microfabrication; Microneedle; Minimally invasive injection; Skin; Tissue flow conductivity; Transdermal drug delivery

1. Introduction

Microneedles have been proposed as a novel drug delivery method that can capture the convenience of a transdermal patch and the efficacy of a hypodermic needle [1,2]. These microscopic needles have been fabricated by adapting the tools of the microelectronics industry to penetrate typically hundreds of microns into the skin in a painless manner. Solid microneedles have been used to pierce the skin, after which a patch can be applied for passive or iontophoretic delivery across permeabilized skin [3–5]. Solid microneedles have also been coated with drugs, proteins, DNA and vaccines for rapid dissolution within the skin [6,7].

Drug delivery by injection through hollow microneedles has been demonstrated to deliver small (~10 μl) quantities of insulin to animal models [8,9] and even smaller quantities (1 μl) of methyl nicotinate to human subjects [10]. Achieving larger flow rates has been difficult, apparently due to low flow conductivity in the skin. To determine what limits flow into the skin from microneedles, we recently tested the hypothesis that infusion through hollow microneedles into the skin is limited by the resistance to flow offered by the dense dermal tissue compressed during microneedle insertion [11]. Consistent with this hypothesis, our previous study showed that by first inserting microneedles into the skin and then partially retracting before infusing fluid increased infusion flow rate by up to more than a factor of 10. Microneedle retraction similarly increased infusion of an insulin solution to diabetic rats in vivo [12]. In this study, we seek to further test this hypothesis and in particular determine what happens to the skin microstructure during microneedle insertion and retraction.

2. Materials and methods

The materials and methods used in this study have been described previously [11]. In brief, single glass microneedles were fabricated with an effective tip opening radius of 30 μm and a tip bevel angle of 38°. Microneedles were inserted into human cadaver abdominal skin (Emory University Body Donor

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Program, Atlanta, GA, obtained with approval from the Georgia Institute of Technology IRB), which had been stored at −80 °C, warmed to room temperature, hydrated, cut into 4×4 cm pieces, and stretched onto a stainless steel specimen board with eight tissue-mounting pins on it to qualitatively mimic the tension of living human skin.

To image infusion into the skin, a microneedle was inserted using a rotary drilling motion [12] to a depth of 1080 μm into the skin and either left in place or retracted 720 μm back toward the skin surface. A solution of 10^{-3} M sulforhodamine-B (Molecular Probes, Eugene, OR) was then infused into the skin for 104 min at 138 kPa infusion pressure. The top surface of the skin was imaged using bright-field microscopy (Leica DC 300; Leica Microsystems, Bannockburn, IL).

To image microneedle insertion and retraction within the skin, a microneedle was similarly inserted to a depth of 1080 μm into the skin and either left in place or retracted at different distances back toward the skin surface. A small volume of blue–green dye solution (Tissue Marking Dye; Triangle Biomedical Sciences, Durham, NC) was then injected to mark the needle tip location. These skin samples were fixed using paraformaldehyde/glutaraldehyde with microneedles in place [11]. Microneedles were then removed and skin samples were sectioned and examined by bright-field microscopy.

3. Results

Our previous study tested the hypothesis that infusion through hollow microneedles into the skin is limited by the resistance to flow offered by dense dermal tissue compressed during microneedle insertion [11]. Consistent with this hypothesis, partial microneedle retraction out of the skin following needle insertion should relieve tissue compaction and thereby increase infusion into the skin. Indeed, insertion of a microneedle to a depth of 1080 μm without retraction resulted in very little flow into the skin (26 μl over 104 min equals 15 μl/h, Fig. 1B). In contrast, insertion of a microneedle to the same depth, followed by retraction of 720 μm, resulted in extensive infusion into the skin (336 μl over 104 min equals 194 μl/h, Fig. 1C). Our previous study further optimized flow rates after partial microneedle retraction to more than 1.1 ml/h [11]. A separate study showed increased infusion to hairless rat skin in vivo after microneedle retraction [12]. The use of multiple microneedles is expected to increase the flow rates still further.

In order to explain why microneedle retraction has such a large effect on infusion into the skin, we first sought to determine what happens to the skin microstructure during needle insertion. As an indirect assessment, the force applied to the skin during microneedle insertion was measured as a function of microneedle displacement using methods described previously [13]. Just after initial contact with the skin, the force required to move the needle at a needle displacement of 100 μm was 0.6±0.1 N. As the needle translated deeper, the required force steadily increased to, for example, 3.0±0.3 N at a displacement of 400 μm and 7.6±0.2 N at a displacement of 600 μm. This sharp increase in force with displacement would not be expected for piercing penetration through the skin without tissue compression, which should require a relatively constant force. Indeed, force might increase as the needle penetrates deeper into the skin due to increased frictional resistance caused by the increasing needle surface area contacting the skin and the increasing radius of the tapered needle that requires greater tissue displacement. However, the sharp increases in the observed force profile suggest that tissue compression also occurred due to indenting the skin surface during needle translation. This should require a continuously increasing force as compression becomes increasingly difficult while the skin is deformed from its relaxed state.

Skin mechanics during microneedle insertion can be better understood by examining the skin microanatomy after insertion. Fig. 2A1 shows a histological cross-section of human cadaver skin before needle insertion. A thin layer of epidermis (purple) is seen atop a thick layer of dermis (pink). Fig. 2A2 shows human cadaver skin fixed immediately after needle insertion without needle retraction. Significant indentation of the skin is seen. Examination of the magnified view in Fig. 2B2 shows that most of the indented tissue is covered by an apparently intact layer of epidermis and only the lower 100–300 μm of the tissue indentation has penetrated into the dermis (i.e., is not covered by the epidermis). This suggests that during the microneedle insertion of 1080 μm, 800–1000 μm of needle displacement caused tissue indentation and only 100–300 μm caused tissue penetration.

This observation helps explain both the need for “deep” (i.e., >1 mm) microneedle insertion and the reason for the tissue compaction that it creates. Because the skin is highly elastic, pressing a microneedle against the skin initially indents the skin and only after a minimum “insertion force” is achieved does microneedle insertion occur. Thus, even though a microneedle might be displaced more than 1 mm from the initial skin surface, it only penetrates a small fraction of that distance into the skin. In this study, that fraction was approximately 10–30%. Insertion under different experimental conditions would probably lead to different depths of penetration.

This level of tissue deformation should lead to significant tissue compaction. In this study, the edges of the skin were pinned in place under tension and the skin rested on a rigid metal surface. Before microneedle insertion, skin thickness was approximately 2 mm. Skin deformation of close to 1 mm during microneedle insertion should therefore locally decrease skin thickness by ~50%. Because cells and extracellular matrix in the skin are largely fixed in position, such a large change in skin thickness would most likely squeeze out interstitial fluid. Because skin is 60–70% water [14], a halving of skin thickness should require removal of almost all fluid and thereby collapse tissue porosity and effective pore size to almost zero. Changes in porosity, and especially pore size, are known to strongly affect the resistance to flow in porous materials [15]. Consistent with this mechanism, decreased hydration in skin has been specifically shown to reduce flow conductivity [16].

To increase the flow conductivity that was reduced during needle insertion, microneedle retraction is proposed to relieve the tissue compaction. Fig. 2A2–A7 shows histological cross-sections of human cadaver epidermis during needle retraction from an initial insertion depth of 1080 μm (Fig. 2A2) to a final

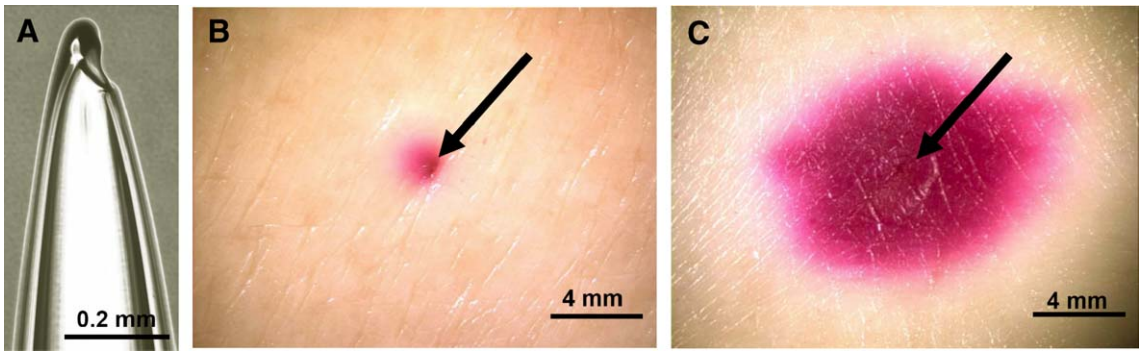

Fig. 1. Top surfaces of human cadaver skin after infusion of sulforhodamine solution using a hollow microneedle (A) in vitro without (B) and with (C) needle retraction (see Materials and methods for experimental condition). Sites of sulforhodamine infusion are indicated by the dark red staining. The site of needle penetration is shown by the arrow. These images are representative of more than 30 similar experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

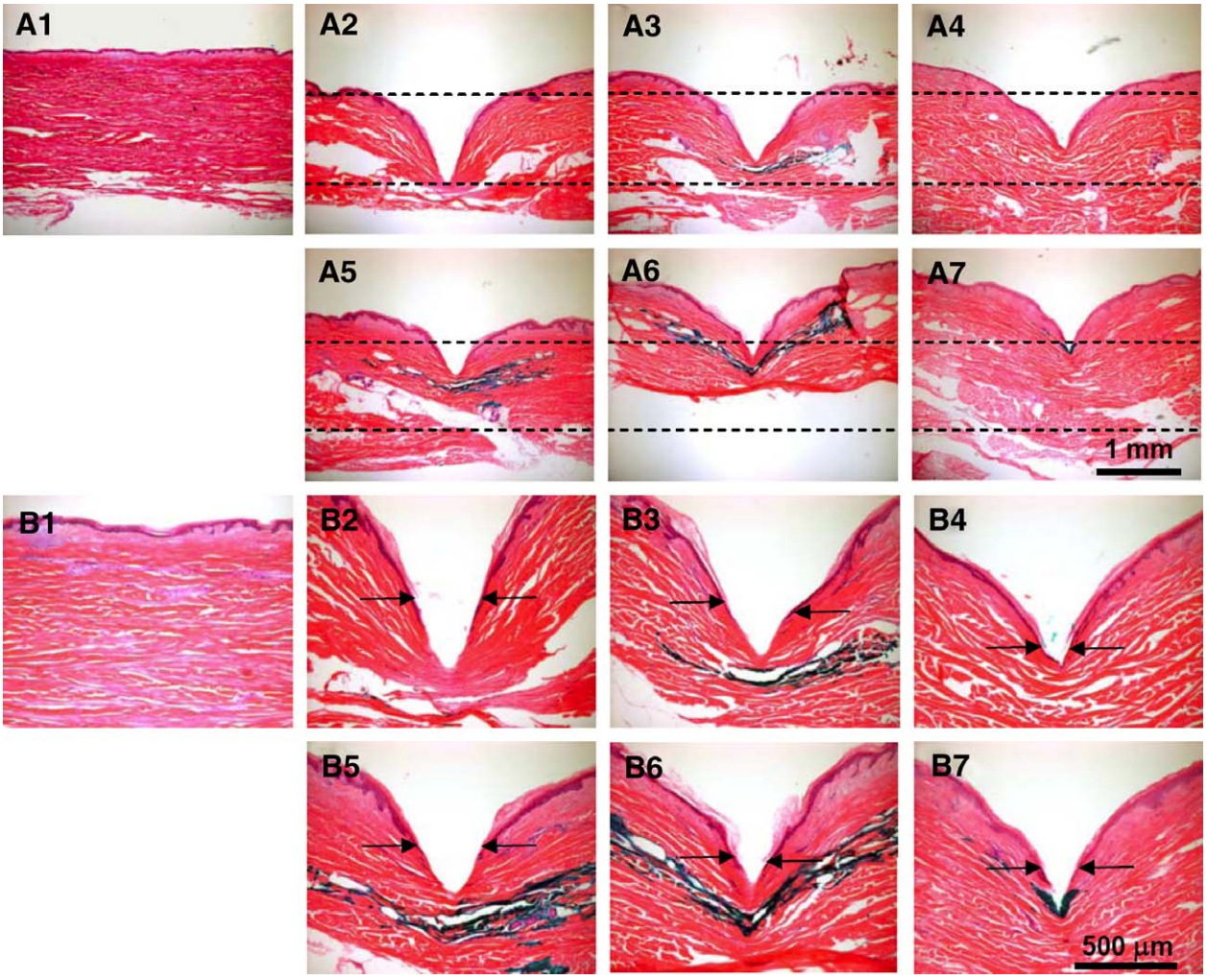

Fig. 2. Histological section of human cadaver skin pierced with a hollow microneedle in vitro shown at low (A) and high (B) magnification. Skin is shown before insertion (1) or after a needle was inserted to a depth of 1080 μm and left in place (2) or partially retracted 180 μm (3), 360 μm (4), 540 μm (5), 720 μm (6), and 900 μm (7) back toward the skin surface. A small amount of blue dye was infused into the skin to mark the needle tip location and then the skin was fixed with the needle in place. Before H & E staining and histological sectioning, the microneedle was removed and is not present in the images shown. The dashed lines in (A) indicate the pre-insertion skin surface location (upper line) and the pre-retraction microneedle tip insertion depth (lower line) estimated by placing the lower line at a distance below the post-retraction needle tip location equal to the retraction distance and placing the upper line 1080 μm above the lower line. The site of needle penetration into the skin (i.e., where the stratum corneum has been breached) is indicated with arrows in (B). These images are representative of at least 3 replicates prepared at each condition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

insertion depth of 180 μm (i.e., retraction of 900 μm; Fig. 2A7). During the retraction process, it appears that the microneedle remained embedded within the skin and that the retraction primarily led to a recoiling of the skin surface indentation back toward and past its original position. Fig. 2A2–A7 shows the relative position of the microneedle penetration moving up from its initial insertion depth of 1080 μm (lower dashed line) toward the skin's original surface location (upper dashed line). The magnified images in Fig. 2B2–B7 show that the geometry of the site of penetration (i.e., as opposed to the site of skin deformation) remains generally unchanged during retraction. Thus, the microneedle tip appears to have remained embedded at a depth of 100–300 μm into the skin at all retraction positions. However, skin thickness beneath the needle tip appears to have steadily expanded during the retraction process, which increased skin water content and porosity/pore size, and thereby increased the flow conductivity. A quantitative measure of skin thickness during retraction is difficult due to the variability in initial skin thickness between the samples and the relatively small sample size ($n=3$ at each retraction depth).

4. Discussion

Injection or infusion into the skin using hollow microneedles is an attractive drug delivery method, but has been difficult to achieve at large flow rates. This study provides an explanation for why infusion through microneedles is difficult, as well as strategies to overcome these difficulties. Because skin is elastic, needle insertion is associated with tissue deformation. The resulting skin compaction locally reduces flow conductivity and thereby makes infusion difficult. This explains why early attempts using microneedles mounted on the end of syringes were ineffective. Pushing harder and harder on the syringe plunger only compressed the skin further and blocked flow. Tissue relaxation by needle retraction is needed to return skin flow conductivity to levels that permit infusion.

Tissue compaction during infusion can be avoided by preventing tissue deformation during insertion and/or relieving tissue compaction afterwards. In this study, microneedles were inserted into the skin using a drilling motion, which was designed to reduce tissue compaction relative to, for example, direct penetration [12]. Despite this approach, significant skin deformation still occurred. Other approaches to reduce skin deformation include insertion using a vibrating motion [12,17] and insertion at high velocity [18]. Tissue compaction was relieved after insertion in this study by partial needle retraction, although other approaches may also be effective.

The increased flow rate after microneedle retraction could also be explained by the formation of a cavity in the skin left behind during needle retraction. However, the observation in Fig. 2 that the skin appears to recoil in concert with the microneedle while the needle tip remains within this cavity during retraction is inconsistent with this hypothesis. Moreover, the total volume of the cavity formed in the skin is on the order of 10 nl, based on the microneedle geometry. This volume is insignificant when compared to the 336 μl delivered in Fig. 1C.

The increased flow rate could also be explained by removing a tissue plug from within the needle bore during microneedle retraction. Because it is difficult to image the needle bore within the skin, direct visual evidence addressing this possibility is not available. However, indirect evidence suggests that tissue does not enter and plug the needle bore. First, microneedles used in this and previous [11,12] studies were beveled, such that the bore opening was offset from the needle tip (Fig. 1A). This decreases the chance of tissue entering the bore. In addition, mechanical studies that measured the force to insert microneedles into the skin showed that insertion force did not depend on needle wall thickness; in fact, there was no difference between insertion of a hollow or solid microneedle with the same outer tip geometry [13]. Because the force of insertion experimentally and theoretically depends on the area of contact between the needle tip and the skin [13], a coring penetration by a hollow needle should require less force (i.e., contact area equals just that of the annulus formed by the needle wall) than a blunt penetration by a solid needle (i.e., contact area equals the full area of the cylindrical face). These force measurements suggest that hollow microneedles behave as blunt-tipped probes that do not core the skin, probably due to the skin's inability to deform with sufficiently small radius of curvature to bend around the narrow wall of a microneedle.

5. Conclusion

This study supports the hypothesis that infusion through hollow microneedles into the skin is limited by the resistance to flow offered by dense dermal tissue compressed during microneedle insertion. In vitro microscopy studies showed that during microneedle insertion, most needle displacement caused skin indentation, while only a small fraction (i.e., 10–30%) of needle displacement was associated with penetration into the skin. This skin indentation locally compressed the skin, which is expected to dramatically decrease skin water content, porosity/pore size and, thereby, flow conductivity. This at least partially explains why infusion into the skin using microneedles is difficult. Partial retraction of microneedles was shown to relax the skin deformation, thereby relieving skin compaction and increasing flow conductivity, while the microneedle tip remained embedded in the skin. Altogether, these findings indicate that the combined approach of microneedle insertion for penetration into the skin and microneedle retraction to relieve skin compaction is an effective approach to infuse fluid into the skin in a minimally invasive manner.

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