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Brief/Technical Note

Impact-Insertion Applicator Improves Reliability of Skin Penetration by Solid Microneedle Arrays

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INTRODUCTION

Microneedles are needle-like structures shorter than 1 mm that have been advocated as devices for enabling potentially pain-free intradermal delivery of biomacromolecules (1–5). To permit a reproducible delivery of the drug, microneedles should be inserted into the skin in a controlled and reproducible manner (4,6). Obviously, one of the factors that influence the penetration ability of microneedles is the insertion process itself, *e.g.*, microneedles can be inserted manually or by using insertion devices (3,4,6–9). Recently, the penetration ability of low-density arrays (42 microneedles/cm^2) with 750-μm long microneedles, applied manually or by using a snap-based applicator, was investigated (10). However, no studies have been reported on the efficiency and reproducibility of the insertion of high-density microneedles into human skin. Therefore, the aim of this study was to investigate the effect of the type of application on *inter* and *intra* individual variability of microneedle insertion into human skin by microneedle users.

We show that participants using an impact-insertion applicator inserted high-density microneedles into *ex vivo* human skin with high efficiency and with a low *inter* and *intra* individual variation. However, when the same microneedle arrays were inserted by using a manual insertion device, the penetration efficiency was reduced by approximately 40% with a considerably lower reproducibility. Finally, the applicability of an impact-insertion applicator/microneedle array was confirmed in a vaccination study in mice.

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MATERIALS AND METHODS

Microneedle Application

In this study, high-density arrays (576 microneedles on a 5× 5-mm backplate, gifted by Bosch, Germany, Stuttgart) with a microneedle length of 200 μm were used (Fig. 1). The microneedles were applied onto *ex vivo* human abdominal skin that was obtained from hospitals within 24 h after cosmetic surgery and dermatomed to a thickness of 600 μm (11). To investigate factors that influence the penetration efficiency, microneedles were applied onto the skin for 10 s with different forces (3.43–22.1 N) by applying weight rods (350–2,250 g) onto the insertion device for manual application. Also, the effect of application time (5–60 s) with a constant force (7.36 N) was investigated. Next, 15 participants (21–57 year; 10 male and 5 female) volunteered to apply a microneedle array three times onto *ex vivo* human skin, in a direction perpendicular to the skin surface, by using either a manual application device (Fig. 1d), or an impact-insertion applicator at a velocity of 3 m/s (Fig. 1e) (12). The type of microneedle application was randomly performed.

Penetration Ability of Microneedles

To determine the penetration ability, 70 μL of an aqueous 0.4% trypan blue (Sigma-Aldrich) solution was applied and left for 1 h at the site of prior microneedle application. Subsequently, the skin surface was washed two times with water and once with 70% ethanol. Next, the stratum corneum (SC) was removed by tape-stripping (Scotch tape) until no SC residue was visually observed on the tape and the skin appeared shiny. Finally, the penetration efficiency (PE) was calculated as follows:

$$PE = (\text{number of blue spots}/576)*100\%$$

Safranin Staining

To visualize the SC removal efficiency, 10-μm thick cryosections of control and tape-stripped skin were made on

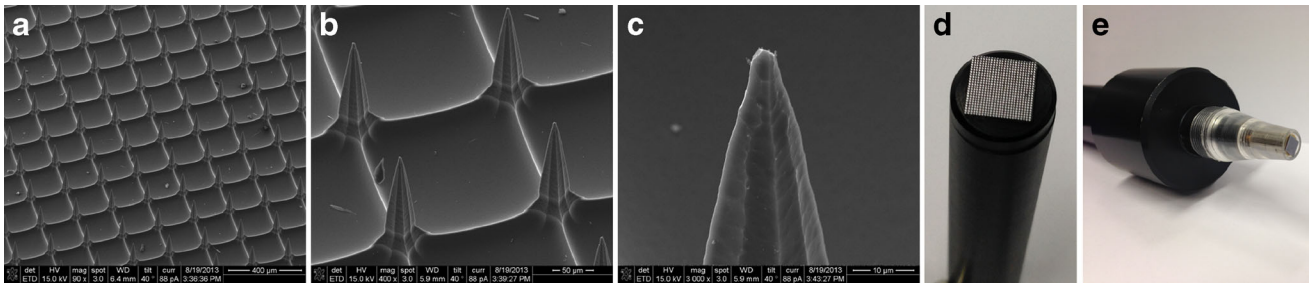

Fig. 1. Electron micrographs of a high-density array of 200-μm long silicon microneedles with a magnification of ×90 (**a**), ×400 (**b**), and ×3,000 (**c**), mounted on a manual insertion device (**d**) or an impact-insertion applicator (**e**)

a Leica cryostat (CM 3050S) and stained for 1 min in a 1% (*w*/*v*) Safranin O solution (Sigma-Aldrich). Next, the number of layers was visualized by swelling the SC in a 2% (*w*/*v*) KOH solution for 20 min (n=3).

Immunization of Mice with Ovalbumin

Eight-week-old female BALB/c mice (Charles River) were immunized thrice with intervals of 3 weeks by the poke and patch approach (100 μg ovalbumin/70 μL PBS (pH 7.4) for 2 h, as previously described (13)), by using the impact-insertion applicator to apply either the high-density microneedle arrays or our first-generation LU-microneedles. The latter are made of 30 G needle tips, 300 μm long, and fixed in a backplate as a 4×4 microneedle array. They were previously shown to penetrate the skin when using the impact-insertion applicator (12). A subcutaneous injection of 5 μg ovalbumin in 100 μL PBS was used as positive control. Ovalbumin-specific serum IgG responses were determined by a sandwich ELISA, as described previously (13). Antibody titers were expressed as the log value of the serum dilution at the midpoint of a complete S-shaped absorbance-dilution curve. The study was carried out under the guidelines complied by the animal ethic committee of the Netherlands, and was approved by the "Dierexperimentencommisie Universiteit Leiden (UDEC)" under number 13065.

RESULTS AND DISCUSSION

Assessment of Piercing Efficiency After Stratum Corneum Removal

The application of dyes at the site of microneedle application is commonly used to assess microneedle

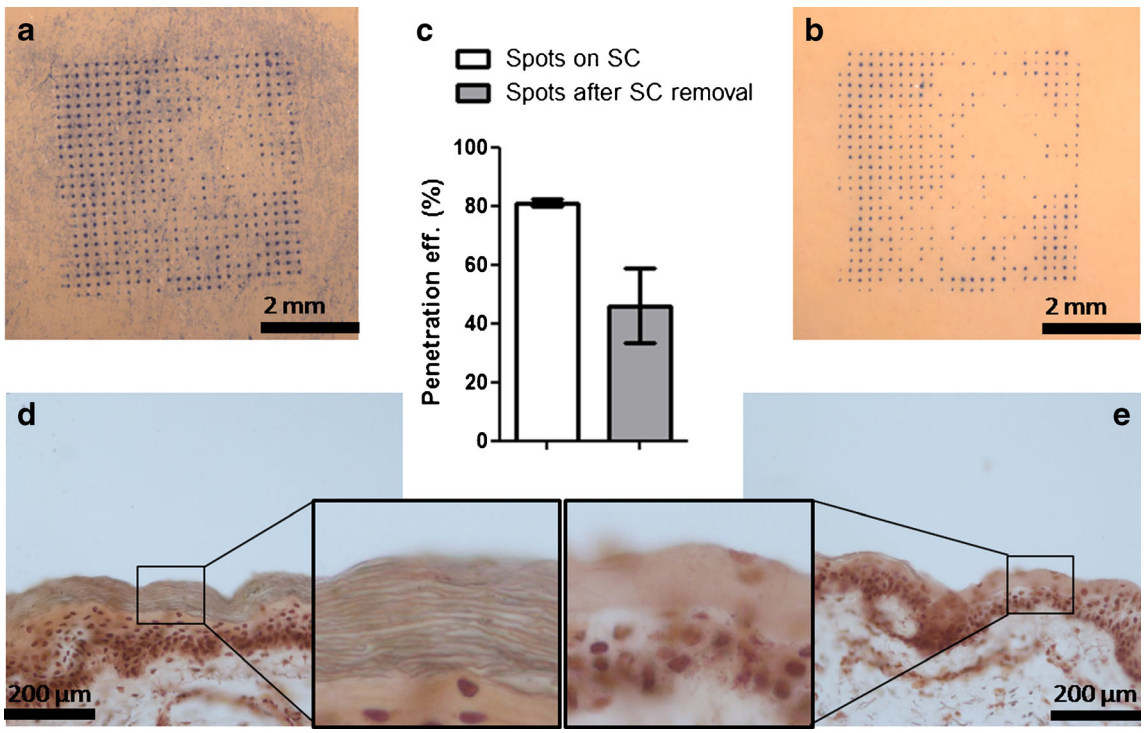

Fig. 2. Representative examples of microneedle-pierced trypan blue stained *ex vivo* human skin before (**a**) and after stratum corneum (SC) removal (**b**). Calculated penetration efficiency before and after SC removal (mean±SD, n=3) (**c**). Representative examples of Safranin O stained freeze coupes of non-stripped (**d**) and tape-stripped (**e**) *ex vivo* human skin

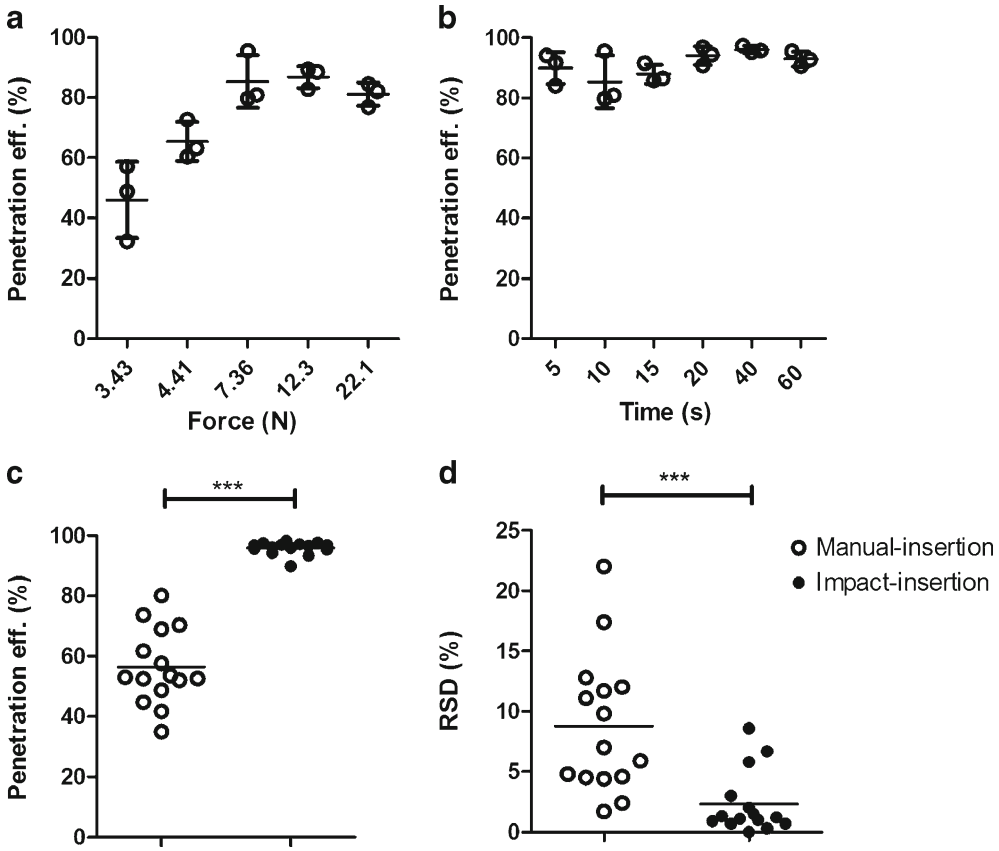

Fig. 3. Penetration efficiency after application of a high-density microneedle array onto *ex vivo* human skin with different forces for 10 s (**a**) and at a constant force (7.36 N) with varying application times (**b**). The application of microneedles onto *ex vivo* human skin by non-experienced microneedle users (**c**) and (**d**). Fifteen participants pierced the skin with 200-μm long microneedles by a manual insertion device (*open circles*) or an impact-insertion applicator (*closed circles*). Each point represents the average penetration efficiency of three individual microneedle applications by one individual (**c**) and the relative standard deviation (RSD) of the penetration efficiency for each participant (**d**). Significance (***$p<0.001$) was determined by an unpaired two-tailed *T*-test

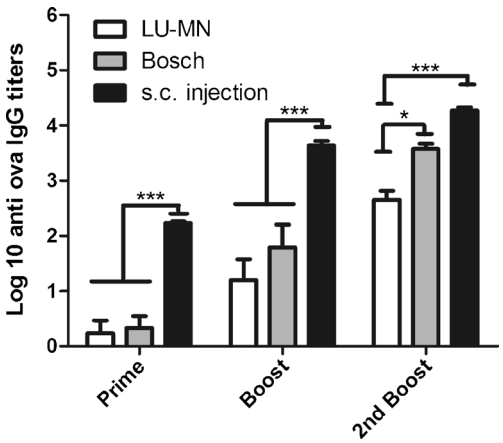

Fig. 4. Ovalbumin-specific IgG responses upon microneedle-based ($n=8$) immunization by using an impact-insertion applicator with first-generation (LU-MN) or high-density (Bosch) microneedles, and subcutaneous (s.c.) injection ($n=5$). Each bar represents the mean± SEM. Non-responders were given an arbitrary titer of 1, and significance (*$p<0.05$, ***$p<0.001$) was determined by a two-way ANOVA with a Bonferroni post test

penetration in the skin (12,14–16). However, when observing the complete skin following microneedle application, the resulting spots are not always penetrations, but could be indentations of the SC (see Supplemental information). Indeed, our study made clear that without SC removal, the penetration efficiency is overestimated. When microneedle arrays were applied with a manual insertion device onto *ex vivo* human skin, blue spots were visible (Fig. 2a), suggesting successful skin piercing with an efficiency of 81% (Fig. 2c). However, after removal of the SC, less blue spots were visible (Fig. 2b), showing that only 46% of the microneedles pierced the skin (Fig. 2a). The SC consists of 20 layers of corneocytes (Fig. 2d), and after tape-stripping, most of the SC was removed (Fig. 2e), which allowed a reliable assessment of the piercing efficiency. Therefore, this approach was used in the study described below.

Penetration Ability of High-Density Microneedle Arrays

First, two factors, application force and time, that potentially influence the penetration efficiency of manual microneedle application were investigated (Fig. 3). Increasing the applied force (at a constant application time of 10 s) up to

7.36 N greatly improved the penetration ability (3A), which is in agreement with the literature (9). Further increase of the force or prolonging the application time of the microneedles at a constant force (7.36 N) only minimally increased the penetration efficiency but improved the reproducibility (Fig. 3b).

Next, to investigate whether persons without microneedle experience are able to successfully and reproducibly penetrate skin, 15 participants were instructed to apply microneedles onto *ex vivo* human skin by using a manual insertion device or an impact-insertion applicator (Fig. 3c, d). Using a manual application device resulted in low penetration efficiencies (56%) and relative high *inter* and *intra* individual variation, which was probably caused by variations in applied force and that the microneedle application conditions were below the optimum insertion conditions. However, when using the impact-insertion applicator, all participants pierced the skin with significantly lower *inter* and *intra* individual variation. These results show that using a microneedle applicator is essential for efficient and reproducible penetration of skin by a high-density microneedle array.

Vaccination Study in Mice

To demonstrate that the use of the impact-insertion applicator can lead to reproducible immune responses following microneedle-mediated immunization, we performed a vaccination study in mice, using ovalbumin as a model antigen. Figure 4 shows that microneedle-based vaccination leads to the induction of reproducible ovalbumin-specific IgG responses. Interestingly, using high-density microneedles resulted in up to ten-fold higher IgG responses as compared to our first-generation LU-microneedles. As both microneedle arrays result in reproducible piercing, the difference is probably caused by differences in microneedle density, number (576 *versus* 16 microneedles/array), and geometry (12). Furthermore, a comparison between microneedle-based and subcutaneous administration revealed that vaccination by using high-density microneedles initially led to significantly lower IgG responses than subcutaneous vaccination, but the differences became negligible after the 2nd boost. This shows that microneedle-based vaccination can lead to comparable immune responses as compared to conventional immunization. Similar or slightly lower responses of microneedle-mediated vaccination with ovalbumin, as compared to subcutaneous/intramuscular injection, has been observed before (17,18). In conclusion, these data show the potential of using high-density microneedles with an impact-insertion applicator for vaccination.

CONCLUSION

This study shows that using an impact-insertion applicator improves the efficiency and reproducibility of high-density microneedle insertion, enabling reliable self-application of microneedle arrays onto the skin. Moreover, it was demonstrated the impact-insertion applicator can be used for microneedle-mediated antigen delivery, yielding robust antigen-specific IgG responses, which depend on microneedle density and/or geometry.

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