Micro and Nano Systems Letters

LETTER

Open Access

Rapid biodegradable microneedles with allergen reservoir for skin allergy test

Le-Giang Tran[1] and Woo-Tae Park[1,2*]

Abstract

With the increasing allergy cases worldwide, this study introduces a biodegradable microneedle system to facilitate allergy testing process. Dissolving microneedle provides a minimally invasive manner to go through skin barrier while avoiding needle phobia among patents, especially children. The microneedles were fabricated using copolymer polyvinylpyrrolidone-co-methacrylic acid (PVP-MAA) material. To ensure the successful insertion of microneedles into the skin, we tailored the mechanical strength of the copolymer by adjusting the weight ratio of two constituted polymers. A reservoir was designed to load allergy specimen for the allergy test. This system is expected to offer a simple and effective allergy testing that can facilitate the allergy testing protocol.

Keywords: Allergy testing, Skin prick test, Microneedle, Polyvinylpyrrolidone, Methacrylate acid

Introduction

As allergy cases are rising worldwide, the demand for rapid and cordial allergy test is high. Currently, skin prick test (SPT) and traditional extract-based allergen-specific immunoglobulin E (IgE) blood test are commonly being used. To perform a SPT, clinician drops allergen extract on the subject's skin and immediately prick the skin with a lancet [1]. Wheals with diameter larger than 3 mm on the application site indicates positive SPT results [2]. Though SPT must be performed by a well-trained clinician, there still are some common false results in the test due to insufficient penetration into the skin and inappropriate pricking location, which is classified as human errors [2]. Therefore, it is important to develop a method to consistently pierce the skin at a controllable depth and pattern.

Utilizing microneedles (MN) as an alternative for lancets could be a promising solution to enhance reliability of SPT. As a mean to penetrate the skin, MNs with size ranging from a few 10 to 100 s of microns were proved to successfully pierce through epidermis layer, the outermost layer of skin [3]. In terms of medical applications, MNs were also demonstrated to be capable of delivering proteins, vaccines and DNA [4–6]. Kang et al. utilized MNs to make micro-incisions in blood vessels for enhanced endovascular drug delivery [7]. This work showed that the conventional drug delivery path can be improved with the assistance of MNs. Additionally, MNs can be integrated in patch, which can have uniform length and distribution. Therefore, MNs are expected to provide an accurate and efficient mean to deliver allergy extracts into the skin. This improves repeatability between tests and reduces human error that can lead to false results.

The concept of using MNs to facilitate allergy testing is proposed in Fig. 1. In this system, a large MN patch contains multiple MN arrays which are loaded with different predetermined allergen extracts. Size and distribution of MNs in the patch should be designed to satisfy the SPT standard. A clinician can effortlessly apply the MN patch to patients all at once. In comparison with conventional SPT protocol, in which a clinician consecutively pricks patient's skin and applies allergy specimen, using MN patch possibly saves time and labor cost while minimizing human errors. Result of the allergy test is then interpreted by wheal size on the skin surface, which can be

*Correspondence: wtpark@seoultech.ac.kr
[1] Convergence Institute of Biomedical Engineering and Biomaterials, Seoul National University of Science and Technology, Seoul, Korea
Full list of author information is available at the end of the article

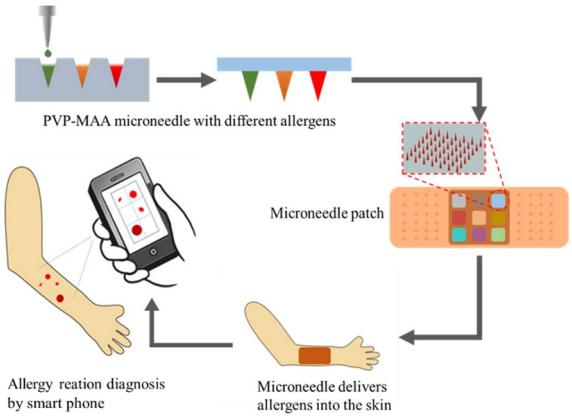

Fig. 1 The concept of using dissolving microneedle patch to facilitate allergy testing

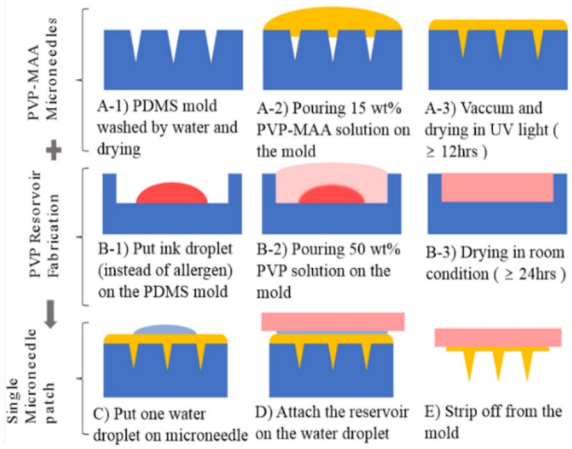

Fig. 2 Fabrication of the polymeric microneedle with drug reservoir

optically measured by a smart phone or similar devices with predetermined criteria. This procedure should still be performed in a hospital setting due to the danger of allergy shock, but this strategy offers the merits of automation and reliability.

This study presents a biodegradable MN patch targeting allergen delivery for the application of allergy testing. Achieving this target faces notable material complication. First, the MN must possess sufficient mechanical strength to pierce the skin. Second, for rapid delivery of allergen into the skin, the biodegradable polymer should have a fast dissolving or degrading rate once inserted in the skin. Furthermore, the fabrication process is required to be opted against extreme conditions as allergen extract might easily degrade. We have designed, fabricated and characterized the microneedle patch. This work does not include the final testing with allergen in live subjects.

Fabrication

The PVP-MAA MN fabrication protocol is depicted in Fig. 2. A 15wt% PVP solution was prepared by dissolving 3 g of 10,000 average MW PVP (Junsei Chemical Co., Japan) in 20 ml distilled water. PVP was selected for its biocompatibility and fast dissolution rate [8]. Moreover, PVP was proved to successfully deliver biological material such as sirolimus drugs [9], encapsulated inactivated influenza vaccines [10], proteins [11], cancer therapeutic agents [12], cosmetic agents [13] and thermal ablation therapy agents [14]. MAA is a nontoxic monomer acid with strong chemical backbone [15]. The solution was magnetically stirred for 1 h at room temperature. Then, MAA (Daejung Co., Korea) was gradually added, and magnetically stirred for 5 h to obtain a homogeneous mixture. The weight ratio of PVP and MAA (100:1, 100:5, 100:10, 100:15, 100:20, 100:25) was prepared. After the copolymer mixture was stable, the resultant was pour into the PDMS mold. The sample was put in vacuum for 30 min to promote infill of all the PDMS mold. Then, the sample was exposed to UV light overnight ($\geq$ 12 h).

In order to fabricate the reservoir, a PDMS block was extruded to the same size of the MN as a reservoir mold. As an alternative of allergen extract, 1wt% Rhodamine B solution was dropped in the mold. Then, 50wt% PVP was poured into the mold and exposed to UV light for crosslinking. Then, the PVP base was adhered to PVP-MAA MNs by adding a drop of water and exposed to UV light. The MN patch was then gently peeled off from the PDMS mold. The optical and SEM image of the fabricated microneedles are shown in Fig. 3.

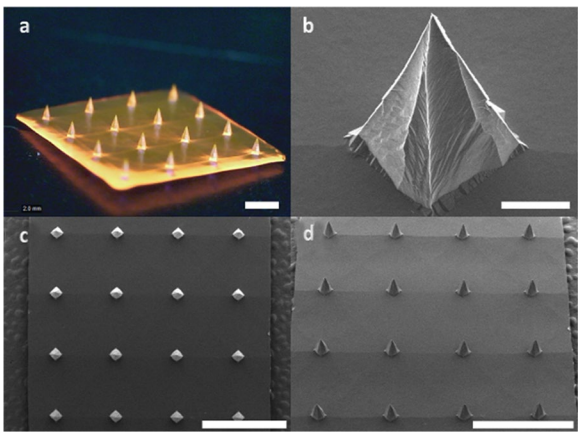

Fig. 3 Images of PVP-MAA MN patch. **a** Optical image of 4 × 4 MN patch containing Rhodamine B (scale bar = 2 mm). SEM images of a single MN (scale bar = 200 μm) (**b**) and 4 × 4 MN patch (scale bar = 2 mm) (**c**, **d**)

Results and discussions

The mechanical test was performed to ensure the MN was strong enough to penetrate into the skin (mainly stratum) with minimum failure. The experiment was setup as follows: A load cell, which is a force measurement unit, was attached to a micro-stage (Fig. 4a). The z-direction micro-stage was controlled by PC interface with the fixed feed rate of 10 µm/s. The load cell was set to vertically compress the MN from top down. The response force was simultaneously collected by Arduino interface. The experiment process was recorded using a micro-camera. The test was performed on MNs with different copolymer compositions.

The function of the microneedle in the skin prick test is to open a pathway in the skin epidermis layer for allergen extracts to enter the dermis layer, which contains blood vessels. Albeit that PVP homopolymer is strong enough to pierce the skin, it is important to tune the mechanical strength of the microneedle for specific needs. MAA monomer was selected to form a copolymer with PVP. MAA was utilized in a variety of biomedical applications owing to its biocompatibility [16]. The incorporation of MAA monomer into PVP matrix induces hydrogen bonds, which strengthen the copolymer matrix [17]. A previous study added MAA as an additive to increase its copolymer compressive strength [18]. As shown in Fig. 4b, the MN with higher MAA ratio induced higher compressive force for the same z-displacement. The experiment process was observed through a camera to ensure that microneedle was vertically pressed (Fig. 4c). When the PVP and MAA weight ratio was 100:25, the reaction force gradually gained 0.1 N for every 10 µm displacement increasement. Data

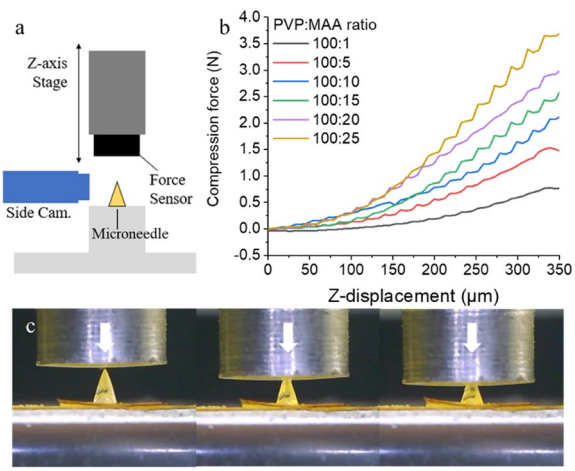

Fig. 4 The experiment setup (**a**) and reaction force (**b**) of the microneedle at different co-polymer ratio. **c** Optical images of the compression process

indicated that MN hardness increased with increasing MAA content. Epidermis is the outermost layer of the skin with average thickness of 200 µm [19]. Therefore, we were interested in z-displacement equal or over 200 µm. The measured compression force at 200 µm z-displacement of 100:15 copolymer was 0.82 N while that of 100:20 copolymer was 1.18 N, which was approximately 1.4 folds. Meanwhile, the different compression force of 100:20 and 100:25 microneedle was insignificant at 200 µm of z-displacement. Therefore, adding MAA into PVP matrix helped improve the mechanical strength of the copolymer structure. Furthermore, it appeared that the reaction force increased steadily with z-displacement, which indicated that there was no major breakage in the MN.

To further verify the insertion of PVP-MAA MN into the skin, we inserted the MN into porcine skin. The 4×4 array MN, which contained 16 MNs, was used in this experiment. The cadaver porcine skin was purchased from local market. Skin was stored in the freezer (− 20 ℃) and defrosted at room temperature for 1 h prior to the experiments. Additionally, the porcine skin was kept moist during the whole experiment section.

The image of the surface of cadaver porcine skin after removing the microneedle is shown in Fig. 5. A successful MN insertion leaved a pierced mark on the skin which can be easily observed. In Fig. 5a, we counted a total of seven pierced marks created by the 100:10 MN array, which accounts for 43.75% successful insertion. In Fig. 5b, the successful insertion rate was up to 81.25% for the 100:20 MN array. In this experiment, though the MN was not significantly damaged during insertion, it sometime could not penetrate through the skin. This characteristic can be explained as the following: first, the force distribution among MN in an array was not uniform, which makes it hard for the MN to pierce the skin. Second, skin disperses the compressive force due to its natural elasticity, thus, preventing it from being pierced.

The ultimate goal is to manufacture a dissolving MN patch that can successfully deliver allergen extract into the skin at controllable doses. Here, we replaced allergens with Rhodamine B for the ease of observation and analysis. Figure 6 shows photographs of the prototype of a dissolving MN patch with Rhodamine B as allergen extract. PVP-MAA MN dissolved rapidly when brought into contact with moisture or water [20]. Therefore, after the MN patch was successfully inserted into the skin, MN started to degrade, resulting in leaving allergen extract into the incision created by MNs. After 10–20 min, allergy response can be interpreted according to the SPT protocol [2].

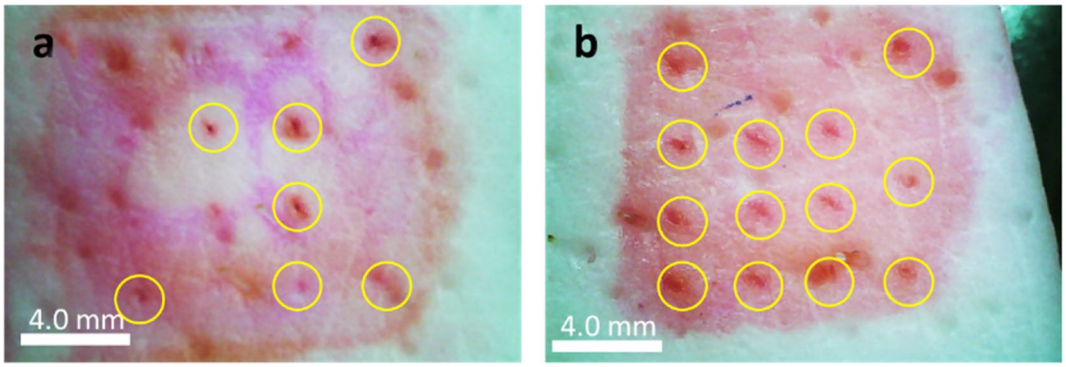

Fig. 5 Images of the cadaver porcine after inserted with PVP-MAA microneedle patch. The weight ratio of PVP and MAA was varied at 100:10 (**a**) and 100:20 (**b**). The successful insertions are marked with yellow circles

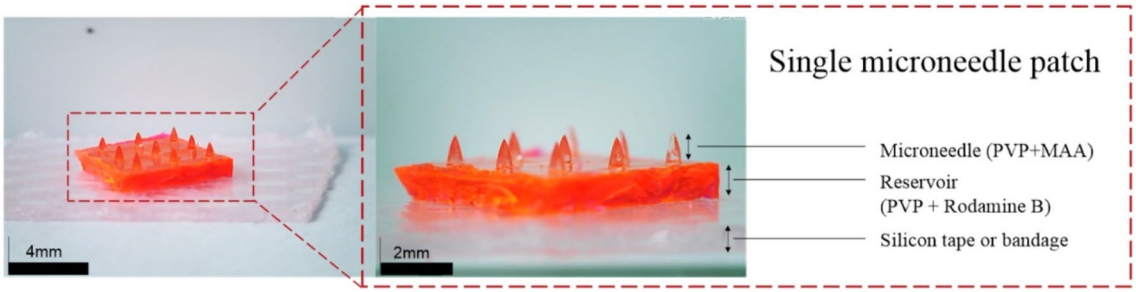

Fig. 6 Images of the biodegradable PVP-MAA microneedle patch with drug reservoir base. Rhodamine B was loaded in the thick PVP-MAA base to resemble allergy extract

Conclusions

In this study, we fabricated a biodegradable MN patch that contains allergen extracts to facilitate allergy testing. PVP-MAA copolymer was selected as the microneedle material owing to it biocompatibility, tunable mechanical strength and fast degrading rate. By changing weight ratio of PVP and MAA in the copolymer, the mechanical strength of MN was increased to sufficiently insert into porcine skin. The dissolving MN patch was designed with a reservoir to simultaneously deliver sufficient amount of allergen extract once inserted into the skin. The MN patch achieved a successful inserted rate of 81.25% in porcine skin. This work is expected to simplify the skin prick test protocol and minimize human errors in the test.

Acknowledgements
The authors thank Prof. WonHyoung Ryu and JiYong Lee for providing the microneedles mold and valuable suggestion on material selection.

Authors' contributions
WTP supervised the findings of this work and reviewed the manuscript. LGT designed, performed the experiments and drafted the manuscript. Both authors contributed to the final manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by the Mid-career Researcher program under the National Research Foundation of Korea (NRF-2017R1A2B4011556).

Availability of data and materials
The datasets supporting the conclusions in this article are included within the article.

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Convergence Institute of Biomedical Engineering and Biomaterials, Seoul National University of Science and Technology, Seoul, Korea. [2] Department of Mechanical Engineering, Seoul National University of Science and Technology, Seoul, Korea.

Received: 12 May 2020 Accepted: 9 July 2020
Published online: 15 July 2020

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