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Nanoparticle popsicle: Transdermal delivery of nanoparticles using polymeric microneedle array

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Abstract−A number of nanoparticles are currently applied for medical or medicinal research fields through conjugating with drugs or bio-molecules such as DNA, RNA, and peptides. There are three distinct ways to deliver nanoparticles into animal and human bodies: injection, feeding, and transdermal administration. We fabricated a polymeric microneedle array carrying nanoparticles on the end-tip of needles for an efficient delivery of functional nanoparticles through the skin. The polymeric microneedles with a length ranging from 600 to 1,000 μm were casted out using laser-printed PDMS (polydimethylsiloxane) molds where the 50 nm silica or polystyrene nanoparticles had been filled by centrifugation. When the microneedle array was applied to the porcine cadaver skin, nanoparticles were quickly and stably dispersed in the hypodermis. Microneedle array conjugated with nanoparticles has a potential for an alternative method to deliver cosmetic or pharmaceutical materials into human skin locally without professional procedures.

Key words: Nanoparticle, Microneedle Array, Polymeric Needle, Drug Delivery

INTRODUCTION

Nanoparticle research has recently attracted attention due to its potential for biomedical, optical, and cosmetic applications. Iron-oxide nanoparticles, for instance, improved the resolution of images taken from ultrasound and MRI due to a better contrast ratio [1] and also were used for enzyme immobilization [2] or pathogen detection [3]. Polymer-based nanoparticles associated with drugs were designed to enhance a pharmacokinetic value, which determines the strength of drug delivery system [4]. Delivery of such nanoparticles to the human body is mostly carried out by hypodermic needle injection; however, the injected nanoparticles frequently accumulate in the liver or kidney resulting in toxicity [5]. Oral delivery of nanoparticle is also problematic because of enzymatic degradation and poor absorption in the human digestive system [6]. A patch type of transdermal drug, which was thought to be an alternative way to deliver drugs in the local area of body, has poor permeability to the stratum corneum [7].

Microneedle arrays were designed to overcome the obstacles induced by hypodermic needles such as pain, bleeding, and skin irritation. Due to the micrometer size of microneedles, both pain and bleeding are efficiently reduced in comparison with conventional needles [8]. The microneedles have been fabricated by carving the needles directly [9] or by filling the mold with substrates [7,10]. The first generation of microneedle was introduced with a similar structure of conventional hollow needles [9] made of rigid metals for the delivery of liquid drugs through their channels. Since the microneedles made of metals had a risky potential to be fragmented in the body or to irritate skin, biocompatible or biodegradable materials such as PVP (polyvinylpyrrolidone), CMC (carboxylmethylcellulose), and agarose were recently used for the fabrication of microneedle arrays [7,10]. Using in situ lens-based lithography, such biodegradable microneedles were produced from the molds fabricated by micro-electromechanical masking and etching procedures [10].

In this study, we fabricated biocompatible agarose and CMC microneedle arrays incorporated with polystyrene or silica nanoparticles in their needles. The microneedles were casted from a PDMS (polydimethylsiloxane) mold prepared by laser writing process directly. Nanoparticles were applied to porcine cadaver skin through those microneedles, and the transdermal delivery of particles was subsequently assessed by analysis of fluorescence from dyes labeled to the nanoparticles.

MATERIALS AND METHODS

1. Preparation of Mold Substrate

PDMS (polydimethylsiloxane, Sylgard 184, Dow corning) substrate for mold was prepared by mixing polymeric PDMS solution (DC-184A) and hardener (DC-184B) with 10 : 1 ratio [11]. The PDMS pre-solutions were poured on flat surface, and then this substrate was kept in a vacuum desiccator to remove blisters completely. Finally, the substrate was heated to be hardened in dry oven at 72 °C for 2 hours before laser etching.

2. Fabrication of Mold Using Laser Writer

The hardened PDMS mold was etched by laser writer to make microneedle array. The programmable CO_2 laser (infrared light) writer (PL-40K, Korea stamp, Korea) was used to make tapered cone holes on PDMS mold. The laser beam was focused with a lens (f=30 mm) onto the PDMS surface. The programmable laser writer could change its power from 40 to 50 Watts and speed for controlling diverse depth, radius and top-edge size of holes [12].

Fig. 1(a) is a schematic diagram for the fabrication of PDMS mold

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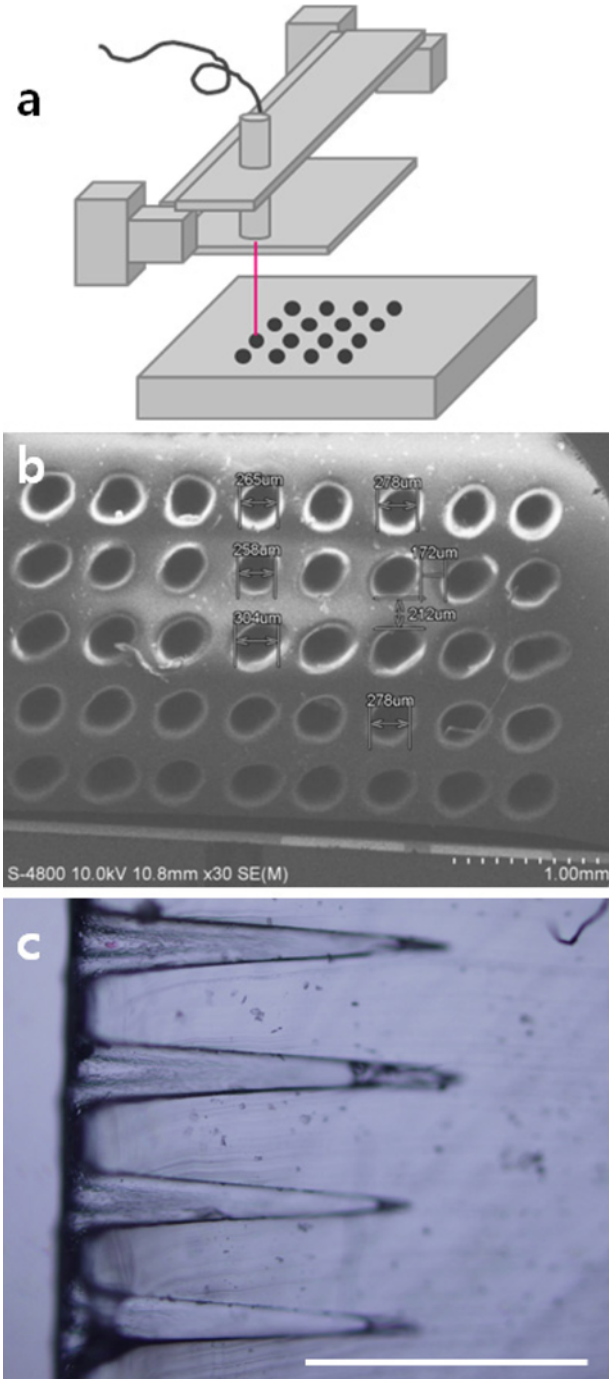

Fig. 1. Fabrication of PDMS mold for microneedle array. Tapered cone holes in the mold were etched by laser beam (a). The range of holes' diameter was from 250-300 μm (b), and the depth of holes was around 1 mm (c). Scale bars are 1 mm.

using laser writer. After laser ablation, molds were washed by ultrasonic cleanser for 10 minutes with acetone and isopropyl alcohol. Each hole for microneedle was patterned into 10 by 10 arrays of 250-300 μm diameters with 400 μm center-to-center space using a drawing program.

3. Fabrication of Microneedle Array Coated with Nanoparticles

Agarose and carboxylmethylcellulose (CMC) were used as substances for microneedle arrays. After agarose powder was dissolved in the 70% aqueous sucrose solution by the ratio of 5 : 100, the agarose mixture was boiled for 20 sec to make agarose solution. The agarose solution was stored in the heat block set at 60 °C for the further casting process. To prepare 5% CMC solution, CMC powder was mixed with deionized water for more than 6 hours at room temperature. After the 5% CMC solution was poured into PDMS mold, a vacuum was applied to fill in the mold holes. To make a nonporous solid microneedle, the mold containing substance was stored in the vacuum dessicator.

Before casting agarose or CMC solution into the mold, nanoparticles were loaded onto the PDMS mold. 2.0×10^{13} particles/ml of 50 nm polystyrene (Polyscience, Warrington, PA) and 1.0×10^{11} particles/ml silica (Biterials, Korea) particles were used as a cargo nanoparticles carried by microneedle array. Both polystyrene and silica nanoparticles have carboxyl group on their surfaces and are labeled with fluorescence dye, Rhodamine and RITC, respectively.

4. Delivery of Nanoparticles into Porcine Cadaver Skin Using Microneedle Array

Microneedle array coated with nanoparticles was pressed on the swine skin for 10 min by finger to deliver particles into the skin. The porcine cadaver skin was purchased from a local butcher shop, and hairs were removed by razor. Since the structure and characteristic of swine skin are similar to those of human, porcine cadaver was thought be an appropriate model for transdermal drug delivery [13,14].

5. Microscopic and Spectrometric Analysis

Optical microscopic images of PDMS molds and microneedle arrays were taken under stereo (Olympus SZ51, Japan) or upright (ZEISS Axio Imager A2, Germany) with objective magnification of 4-100. SEM (scanning electron microscope) images for molds and microneedle arrays were obtained by FESEM (Hitachi S-4800, Japan) with magnification of 30-500. Fluorescence from nanoparticles coated on the needles was analyzed under fluorescence microscope (ZEISS Axio Imager A2) using a Rhodamin shift free filter with magnification of 100-1,000. The fluorescence intensity of nanoparticles detached from mold and swine skin through ultra-sonication wash was assessed by spectrofluorometer (AMINCO-Bowman Series 2, Thermo, USA).

RESULTS AND DISCUSSION

1. Fabrication of Microneedle Mold

The mold for microneedle array was fabricated by laser etching (Fig. 1(a)) on the cross-linked PDMS substrate. The mold contains 100 tapered cone holes whose width and depth were 250-300 μm and 1,000 μm, respectively (Fig. 1(b) and (c)). To obtain smoothly ablated micro-holes in the mold, the power and focus of laser beam was controlled using the computer program connected to the laser writer. The holes with different sizes of depth could be generated by controlling the moving speed of laser beam; for instance, the holes with 1,550 μm, 542 μm, and 270 μm of depths were produced by 1 cm/s, 5 cm/s and 10 cm/s moving speed of laser beam, respectively [11]. Since conventional microneedles have been fabricated through the photolithography and etching to generate a cylindrical shaft, an additional process is required for making tapered or beveled shape [10]. However, this laser writing method enables PDMS

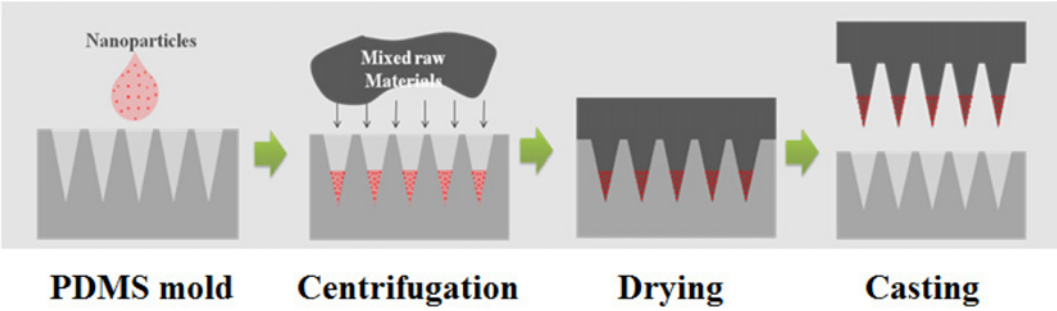

Fig. 2. Schematic diagram for fabrication of microneedle array coated with nanoparticles. After nanoparticles were moved to the bottom of each hole in the mold by centrifugation, polymeric solution containing microneedle substrate was loaded onto the mold. Microneedle array was dried and then casted from the mold after centrifugation.

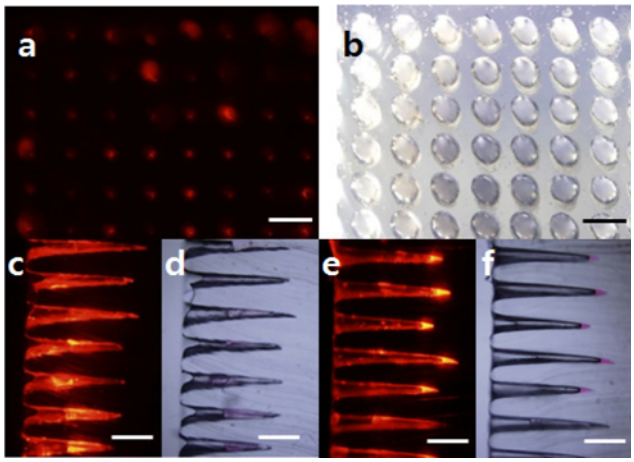

Fig. 3. Localization of nanoparticles in the holes of mold. Nanoparticles were mostly found in the holes (a) rather than out of holes and localized at the bottom of holes (e) by centrifugation before drying, while most of particles were detected in the middle of holes without centrifugation (c). Scale bars are 500 μm.

substrate to contain tapered holes as well as sharp tips just by a one-step fabrication process.

2. Fabrication of Agarose Microneedle Array Coated with Nanoparticles

Nanoparticle-coated microneedles were fabricated by casting agarose-dissolved water in the PDMS mold after dropping polystyrene nanoparticles into the mold (Fig. 2). By the centrifugation after loading nanoparticles onto top of the mold, most of particles were moved into the holes (Fig. 3(a)) and localized at the bottom of holes (Fig. 3(e)). Since nanoparticles were labeled with red fluorescence dye, the localization of particles in the mold holes was analyzed under the fluorescence microscope.

The microneedle array comprising 100 needles in 0.25 mm^2 was casted from the PDMS mold containing polystyrene nanoparticles (Fig. 4(a)), which were successfully coated on the top of needles (Fig. 4(b) and (c)). Since another centrifugation was carried out after loading agarose substrate to the mold, nanoparticles were efficiently moved down and localized at the bottom of holes. Since the longer length of needle has more opportunities to be bent during the casting, we optimized the length of needles at 600-700 μm. Although the depth of holes in the mold was around 700 μm, the length of needles

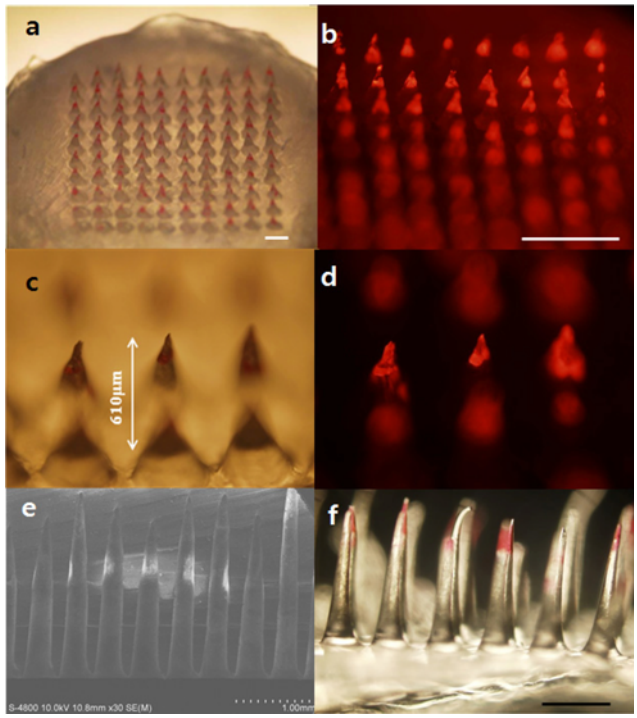

Fig. 4. Microneedle array coated with nanoparticles. 100 agarose needles were arrayed on the 0.25 mm^2 area (a) with a length around 600 μm (c). Polystyrene nanoparticles were coated on the tip of most needles (b), (d). Silica nanoparticles were also coated on the microneedles made of CMC (e), (f). Scale bars without specific note are 500 μm.

was 600 μm due to the shrinkage of agarose substrate during the drying.

For the hardness of needles, sucrose was supplemented into the agarose substrate solution. Without sucrose, it was observed that microneedles were physically deformed and twisted after drying microneedle array in the oven, while the needles were rigid and robust when they were casted from the substrate containing 70% sucrose (data not shown). We found that another reason to make needles deformed was due to the vaporization of moisture from microneedle array. To reduce the deformation of needles, the humidity was controlled around 60% during and after the drying.

Fig. 4(e) shows an SEM image of microneedle array made of 5% CMC (carboxyl methylcellulose) in deionized water. CMC has

been widely used in the food industry and medicinal research due to its biocompatible and biodegradable properties [15,16]. Silica nanoparticles were coated onto this 400 CMC needles (Fig. 4(f)) in an area of 1 cm^2. Those results demonstrate that the proposed fabrication method to incorporate nanoparticles with microneedles is generally used in various substances of nanoparticles and microneedles.

When the microneedle topology was analyzed by SEM images, needles carrying nanoparticles showed greater roughness on their surfaces as well as tips (Fig. 5(a) and (b)), while no rough surfaces and tips were found in the needles without nanoparticles (Fig. 5(c) and (d)). Since there are nanoparticles between inner surface of mold hole and outer part of agarose needle substrate, the rough topology of needle was generated in spite of complete casting and drying.

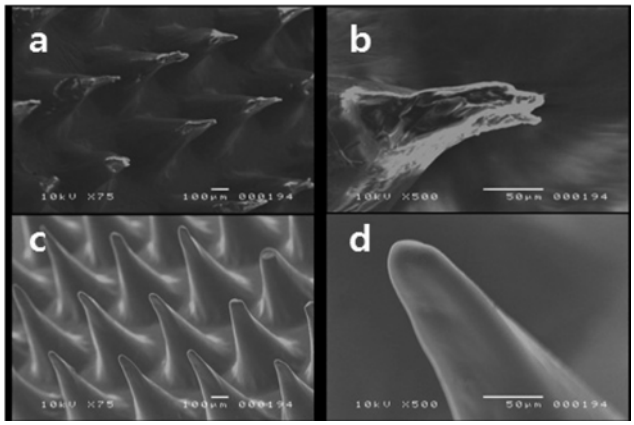

Fig. 5. Topology of microneedle end-tip incorporated with nanoparticles. A roughness was found on the end-tip of needle with nanoparticles (a), (b) by SEM images, while no roughness on the needles without particles (c), (d).

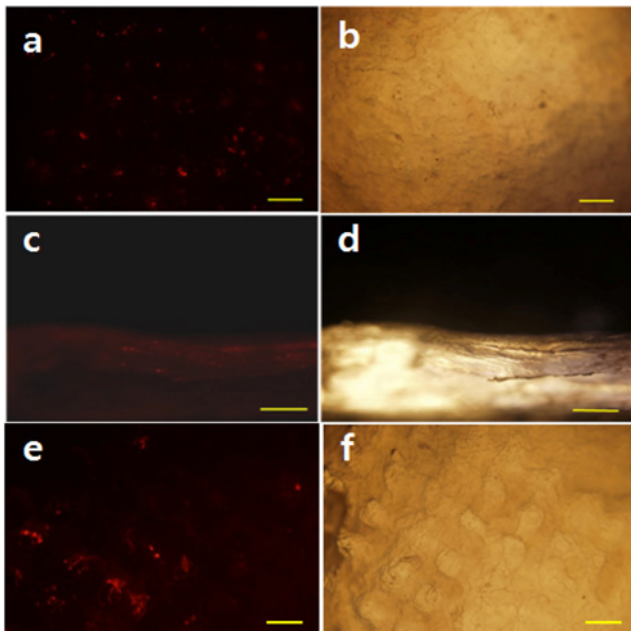

Fig. 6. Transdermal delivery of nanoparticles through microneedle array. Nanoparticles were detected on the surface (a) and in the section (c) of porcine cadaver skin after pressing microneedle array on the skin for 1 min. Particles were stably found on the skin surface 12 hours after detaching microneedle array from skin (e). (b), (d), and (f) are light microscopic images for (a), (c), and (f), respectively. Scale bars are 500 μm.

3. Transdermal Delivery of Nanoparticles Using Microneedle Array

Porcine cadaver skin was used for assessing whether nanoparticles were efficiently penetrated into the epidermal tissue. Nanoparticles were released from the needles and attached to the skin surface (Fig. 6(a)) by pressing the microneedle array with a finger for 5 min. When the distribution of nanoparticles was analyzed by using the sectional surface of porcine skin, the particles were detected at 400 μm below the outer skin surface (Fig. 6(c)). Given that the depth of human epidermis is 100-200 μm, nanoparticles are able to reach to the dermis as well as epidermis by the help of microneedle array.

Nanoparticles penetrated into the porcine tissue were observed in spite of washing in PBS buffer at 12 hours after attaching microneedle array to the skin (Fig. 6(e)). Many research groups modify pharmaceuticals to enhance their stability or pharmacokinetic value in vivo using nanoparticles [17,18]. The proposed popsicle way of using microneedle array coated with nanoparticles has a potential to deliver nanoparticle-associated drugs into the body with greater stability.

4. Efficiency of Nanoparticle Adsorption and Release

We investigated how efficiently nanoparticles were attached to

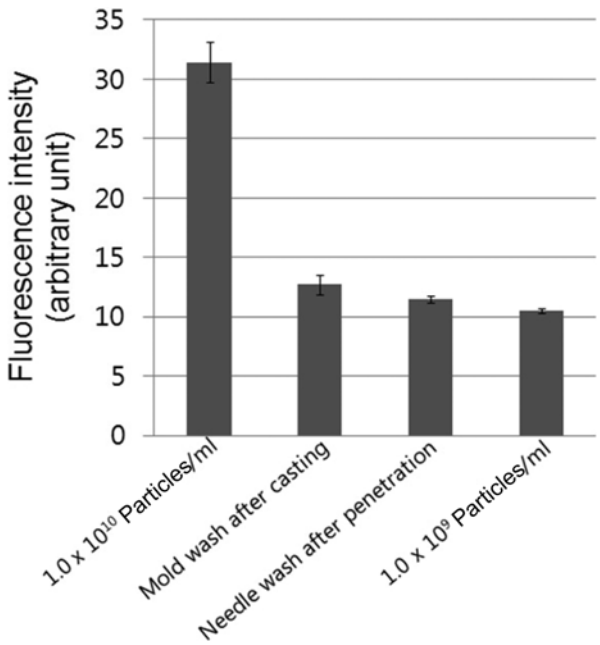

Fig. 7. Efficiency of nanoparticle adsorption and release. Since fluorescence intensity of particles remained on the mold after casting was similar to that of 1.0×10^9 particles/ml, over 99.9% of initial polystyrene nanoparticles (2.0×10^{13} particles/ml) were coated on the surface of agarose needles from the PDMS mold. Most of particles (>99.9%) were also released into the porcine cadaver skin from microneedle array because the fluorescence intensity of particles on needles after penetration is similar to that of 1.0×10^9 particles/ml. Error bars stand for standard deviation.

the microneedle substrates from the molds by measuring the fluorescence intensity using a spectrofluorometer. The fluorescence intensity of particles detached from the mold by washing using an ultrasound sonicator after casting a microneedle array was 12.6 A.U. (arbitrary unit), which is far lower than that of 1.0×10^{10} particles/ml (31.5 A.U.) (Fig. 7). Given that the initial concentration of polystyrene particles loaded onto the mold was 2.0×10^{13} particles/ml, over 99.9% of particles were attached to the needles during the casting. The efficiency of nanoparticle release from needle to swine skin was also evaluated by measuring fluorescence intensity of particles harvested from a used micro needle array by washing after applying it to the skin. The intensity of particles in washing solution was similar to that of 1.0×10^{9} particles/ml (Fig. 7), which indicated that over 99.9% of nanoparticles were released into the porcine cadaver skin through the microneedle array. The holes on the skin surface by needle penetration were not found from the SEM images (data not shown), which suggested that nanoparticles were not transported into dermis through the holes made by microneedles. In the previous study, a cucumber leaf, however, was pierced by CMC microneedles, and thus 20 μm diameter holes were found on the surface of leaf [19]. Although the mechanism of nanoparticle penetration into the skin should be further studied, microneedles were found helpful to deliver nanoparticles to the hypodermis free from wound and pathogen infection through the holes. Since the concentration of nanoparticles was also simply controlled in this encapsulation method, an appropriate and safe dose of nanoparticles may be efficiently delivered into the dermis. This high fidelity of efficiency in nanoparticle coating and delivery demonstrates that the proposed fabrication method to coat nanoparticles on the microneedles is very beneficial for delivering nanoparticles into the hypodermis.

CONCLUSION

Biocompatible and biodegradable microneedle array carrying nanoparticles was easily fabricated using a mold etched by laser writer. 50 nm polystyrene and silica particles were efficiently coated onto the end-tip of agarose or CMC microneedles in the PDMS mold just by centrifugation and drying incubation. When those microneedle arrays were applied to porcine cadaver skin, the nanoparticles were successfully delivered into the hypodermis and stably localized for tens of hours. Since the efficiency of nanoparticle adsorption to the microneedles and the release ratio from needle to skin were over 99.9%, the concentration or dose of nanoparticles was thought be controlled by the purpose of usage.

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