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Applying ceramic nanoporous microneedle arrays as a transport interface in egg plants and an ex-vivo human skin model

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ABSTRACT

Nanoporous microneedle arrays from Al_2O_3 were fabricated via a micromolding process using a PDMS mold generated via a double replication process from a SU-8/Si-master as a template. Hg-porosity measurements showed that the porosity obtained was a function of the temperature used for sintering, resulting in an average pore diameter of 80 nm between 1300 and 1500 °C. Using egg plants it was shown that these nanoporous needles allowed both the delivery of substances, and the extraction of compounds. Subsequently, the delivery of compounds has been evaluated in an ex vivo human skin model using a microneedle array saturated with a labeled monoclonal against a specific marker, DC-SIGN, which is representative for dendritic cells when activated by an antigen. By the latter, it was demonstrated that ceramic nanoporous microneedle arrays are potentially useful for the delivery of vaccines.

1. Introduction

Microneedles for biomedical applications are a field of interest for both the delivery of bioactive molecules to and the sampling of substances from the upper layer of the skin. The clear advantage of microneedles is their painless application, allowing for the delivery and extraction of molecules of interest with minimal trauma [1].

Several techniques are available for the production of microneedles. Micromachining of e.g. silicon usually results in a backplate of the same material with microneedles integrated in an out-of-plane construction [2]. These microneedle arrays (MNA) can be solid or contain a channel, in which case the MNA can function as a miniature hypodermic needle for both the delivery of substances and the extraction of interstitial fluids [3–5]. Solid MNAs are suitable for the delivery of bioactive compounds via coating of the needles with the substance of interest, usually by incorporation of the compound in a biocompatible biodegradable coating [6]. Using the skin-route targeting the upper layer of the skin for vaccination could allow for a dramatic reduction in the amount of compounds needed for an appropriate immune response [7].

Polymer microneedles are also available as a more cost effective approach to manufacture and are often the system of choice for cosmetic applications. Solid microneedles may be also used to increase the permeability of the skin applying the needles, e.g. in form of rollers. Subsequently, formulations can be applied to the pretreated skin area by special leak-tight containers or creams [8].

Although micromolding of ceramic structures has been used in other fields of applications, providing a route for low-cost manufacturing, MNAs synthesized using this technique were not available until recently [9,10].

Ceramic MNAs as developed at the University of Twente, consist of nanoporous alumina, and are produced by micromolding using a silicone rubber (PDMS) mold and subsequent ceramic sintering. The PDMS production mold is manufactured using a micromachined SU-8/Si-master via double replication [10].

In this paper micromolded nanoporous MNAs consisting of Al_2O_3, are characterized. The porosity of the device material was determined using Hg-porosimetry. The nanoporous microneedles were further evaluated for their ability to extract fluidic compounds by using an egg plant and deliver a monoclonal antibody by using an explant model for the investigation of transport through the stratum corneum of human skin [11].

2. Materials and methods

2.1. SU-8/Si-master and PDMS production mold fabrication

For the production of ceramic MNAs, a poly(dimethyl siloxane) (PDMS) mold was used that was generated via double replication

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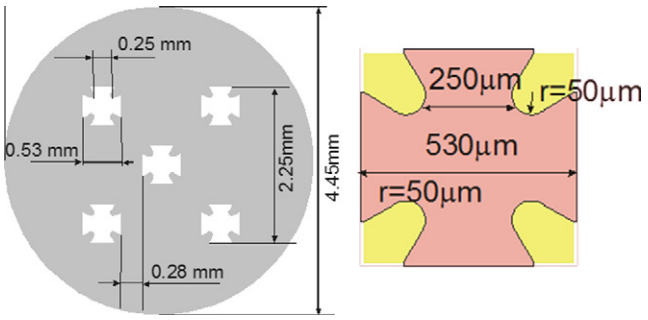

Fig. 1. Mask layout for MNAs.

of a micromachined SU-8/Si-master. The full process of device fabrication has been described by us previously and is here therefore only given in brief [10]. A Si-wafer was patterned using photolithography followed by anisotropic etching through a silicon nitride mask. The silicon nitride layer then is stripped and a conformal thin layer of titanium is deposited and silisidized. Subsequently, thick-film SU-8 photolithography is performed on the pre-patterned silicon wafer to create a modular SU-8/Si-master. The master was then used to generate the 1st PDMS microstructure that, after coating with a fluorocarbon (FC) layer by plasma deposition, formed the template for the final PDMS production mold.

2.2. Ceramic micromolding

For ceramic casting, a slurry made of 43 wt.% alumina (AKP 30, Sumitomo) suspended in 46 wt.% ethanol containing 6.4 wt.% poly(vinyl butyral) (B-98, Tape Casting Warehouse) as a binder and 2.8 wt.% butyl benzyl phthalate (S-160, Tape Casting Warehouse) as a plastisizer, 1.0 wt.% caolin as sintering agent and 0.8 wt.% Solsperse 20000 (Noveon Division Lubrizol Ltd.), was used as a dispersant.

PDMS molds were filled with the slurry, air bubbles removed by treating the mold in an ultrasonic bath, and the slurry was allowed to air-dry. After drying, the ceramic green body was released from the PDMS mold and MNAs with a diameter of 4.5 mm were cut out with a stencil tool. The MNA green bodies were then sintered in a controlled manner utilizing a tube furnace at a maximum sinter temperature of 1500 °C [10].

Ceramic samples for the porosity measurements were prepared in the same way but molded on a flat piece of PDMS for reasons of convenience in the manufacture of test samples. The maximum sinter temperatures were 1200, 1300, 1400 and 1500 °C, respectively.

2.3. Hg porosimetry of ceramic samples

Ceramic samples without microneedles sintered at different temperatures were submitted to Hg-porosity measurements to assess pore size distribution and porosity, using the Hg-Poremaster 60 from Quantachrome Instruments.

2.4. Sampling and delivery of substances using MNAs

To test the hypothesis that MNAs can be used to sample interstitial fluids, a purple egg plant (*Solanum melongena*) was used as a simplified skin model.

To test the ability of MNAs to deliver substances to the skin, an MNA was saturated with a solution of toluidine blue O (Sigma–Aldrich), 20 mg/ml deionized water and pressed onto the skin of a light green Thai egg plant. Visualization of the dye transfer was done using a standard stereo light microscope.

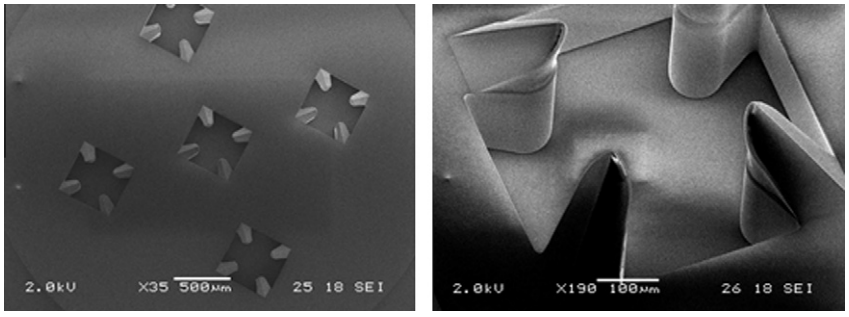

Fig. 2. Scanning Electron Micrographs of 1st PDMS microstructure. Top view of an array (left) and detailed view on the microneedle tips (right).

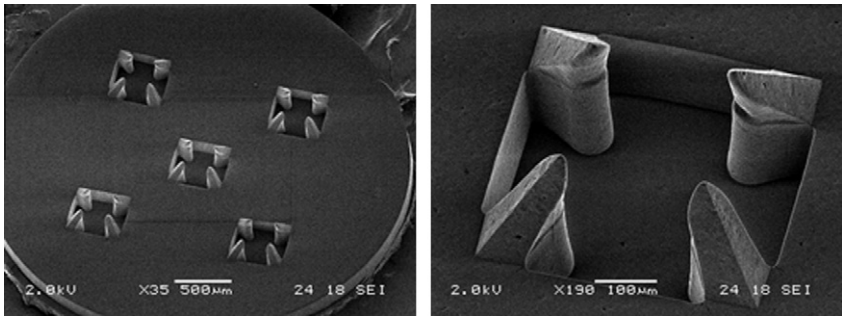

Fig. 3. Scanning Electron Micrographs of a ceramic microneedle array. Overview of the sintered ceramic array also showing the edge cut by the stencil tool (left) and detailed view on the microneedle tips (right).

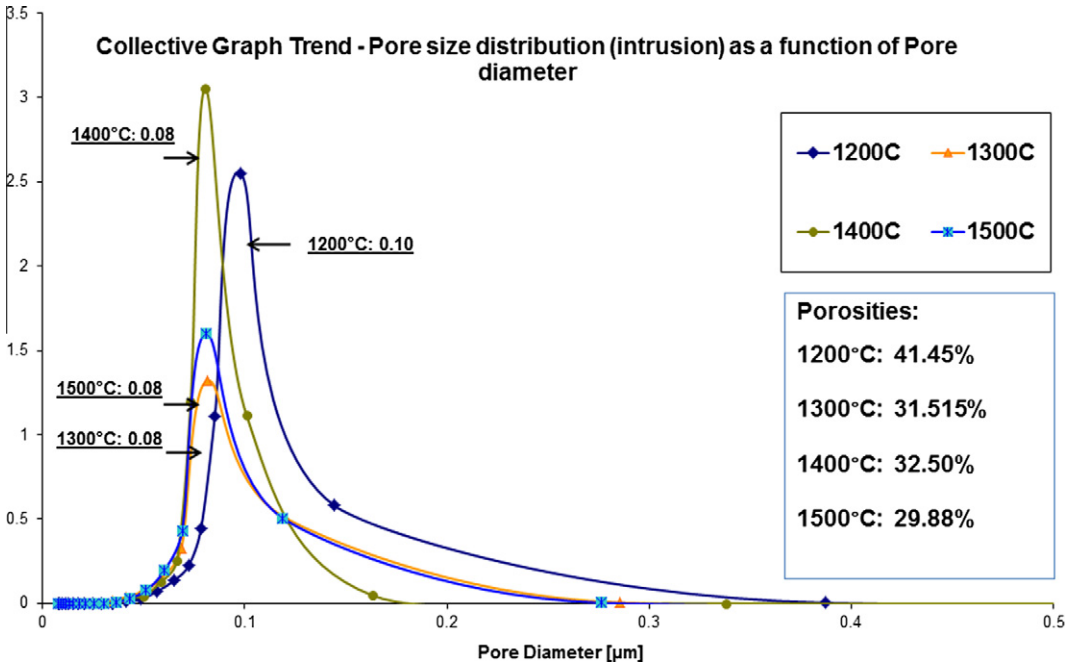

Fig. 4. Pore size distribution as a function of pore size for ceramic samples sintered at different temperatures. Note the decrease in porosity when increasing the sinter temperature.

2.5. Delivery of labeled antibodies to donor skin using MNAs

The fluorescently labeled antibodies (mAbs), DC-SIGN-FITC and anti-DC-SIGN-PE were from Beckton Dickinson Biosciences. MNAs saturated with a solution of DC-SIGN-FITC were pressed onto human donor skin for 10 min. Biopsies were cultivated for 2 days followed by analysis with flow cytometry using a FACSCalibur and CellQuest FACS analysis software (BD Biosciences). The ex vivo human skin procedure has been developed to study novel mechanism for vaccine therapy and is here utilized for a preliminary investigation of MNA performance [11].

3. Results and discussion

Nanoporous arrays consisting of a backplate with seamlessly integrated needles were obtained using a ceramic micromolding process as described by us previously [10]. A variety of microneedle tip shapes, arrangements and porosities can be realized.

Here, we evaluated MNAs of specific geometries, of which one of the mask layouts is depicted in Fig. 1. Varieties of designs have been fabricated, whereas the maximum number of needles in the array tested here for delivery was an array with 64 needles (layout not shown). The tip shape can be modified in the mask layout easily e.g. by changing the parameter r in Fig. 1 (right).

Realizing an SU-8/Si-master as described in Section 2.1. the micropattern was transferred by soft lithography into a 1st PDMS microstructure, which is depicted in Fig. 2. Subsequently, the 1st PDMS microstructure was transferred into the final PDMS production mold used for replication of ceramic microneedle arrays according to the process described in Section 2.2. An example of the latter is presented in Fig. 3. It is remarkable that detailed structural deviations, which originate from the lithographic process in SU-8 are accurately transferred not only into the PDMS microstructure (as expected) but also to the sintered ceramic micropart.

In Fig. 4 the pore size distribution as a function of pore size for ceramic samples sintered at different temperatures is depicted.

At a sinter temperature of 1200 °C ceramic samples with the highest porosity, 48%, and a mean pore diameter of around 100 nm were obtained. However, the arrays sintered at this temperature possessed mechanically weak structures. Exposure to

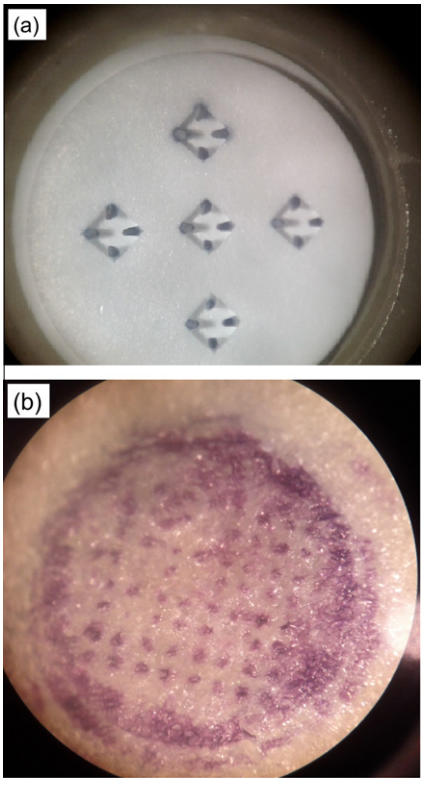

Fig. 5. A photograph of an MNA used to pierce the skin of a purple egg plant. Note the purple (dark colored) needle tips (a). A photograph of egg plant pulp stained with toluidine blue O released from a saturated MNA with 64 needles piercing the skin of the fruit (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

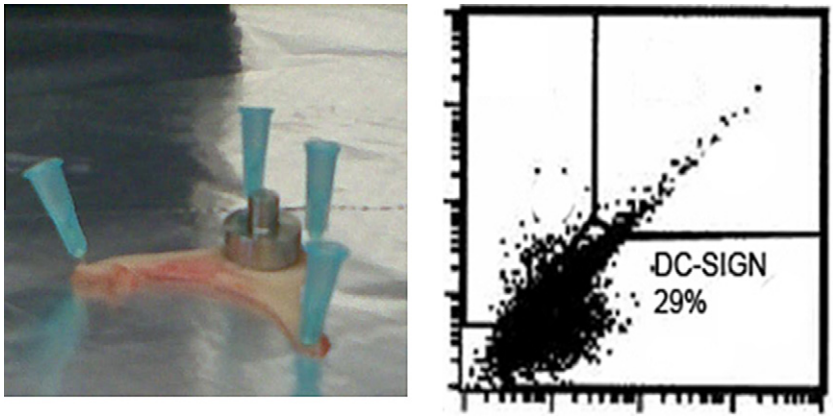

Fig. 6. Donor skin treated with an MNA loaded with labeled antibodies followed by flow cytometry analysis of dendritic cells.

ultrasonic treatment resulted in disintegration of the arrays. Increasing the sintering temperature significantly improved the mechanical integrity. From 1300 °C on the samples were well sintered and showed good mechanical stability.

For sinter temperatures of 1300, 1400 and 1500 °C the porosity values change only marginally and remain around 30% with a mean pore diameter of around 80 nm. This value of the pore diameter is slightly lower than at 1200 °C, however due to higher mechanical integrity that these samples demonstrate we selected 1500 °C as the preferred sinter temperature for MNAs. Therefore, all MNAs for delivery and sampling experiments were sintered at 1500 °C.

An MNA was applied to a purple egg plant (*S. melongena*). During puncturing of the skin cells containing anthocyanins were damaged, releasing the dye that was adsorbed by the porous needles of the array [12]. In Fig. 5(a) the purple color contained in the egg plant skin is uniformly visible in each of the needle tips of the array against the white color background of the ceramics by optical microscopy.

Oppositely, Fig. 5(b) depicts an optical microscopy image of a biopsy taken of a light-green Thai egg plant after pressing a toluidine blue O-loaded MNA against its skin.

Both, these experiments show that it is possible to sample and deliver just below the skin of an egg plant and that the nanoporous microneedles can be used as a breaching device.

Usually, the standard procedure for vaccination is the intramuscular injection of a relatively large volume (i.e. 0.5 ml). A procedure easy to perform, but with a relatively large amount of active substance needed. Microneedle arrays aim at delivery of the vaccine there, where it is most effective: the upper layer of the skin. It is here, in the upper layer of the dermis, and just below the epidermis, where Dendritic Cells (DCs) roam in order to protect the organism against invading pathogens, that enter the body via a damaged skin. DCs process antigen material and bind it to their surface followed by migration to the lymph nodes. Here, the antigen material is contacted with B- and T-cells, initiating an immune response.

Therefore, we expanded the initial egg plant study and also show the transport of fluorescently labeled antibodies (mAbs) to the dermis by pressing mAbs-loaded MNAs onto human donor skin (Fig. 6, left). Explants were taken and cultivated and 2 days later migrated Dendritic Cells (DCs) were analyzed by flow cytometry [11]. Scatter images reveal preferential targeting of the dermal DC marker DC-SIGN, see Fig. 6 (right). Post staining analysis revealed the intradermally targeted DCs to be fully activated.

4. Conclusion

Egg plant experiment have shown that ceramic nanoporous MNAs allow for breaching a skin-type material barrier for sampling or delivery of fluidic compounds. The results motivated us to apply the arrays for the delivery of active compounds to human skin.

We pre-loaded nanoporous MNAs with mAbs solution and pressed the devices subsequently onto human skin. The human skin explant model demonstrates that the microneedles can be also used as a breaching device for preferential targeting the dermal DC marker DC-SIGN in human skin.

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