Journal of Controlled Release 209 (2015) 272–279

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Impact insertion of transfer-molded microneedle for localized and minimally invasive ocular drug delivery

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ARTICLE INFO

Article history:
Received 11 October 2014
Received in revised form 26 March 2015
Accepted 28 April 2015
Available online 30 April 2015

Keywords:
Microneedle
Transfer molding
Impact insertion
Drug delivery
Mouse cornea
Angiogenesis

ABSTRACT

It has been challenging for microneedles to deliver drugs effectively to thin tissues with little background support such as the cornea. Herein, we designed a microneedle pen system, a single microneedle with a spring-loaded microneedle applicator to provide impact insertion. To firmly attach solid microneedles with 140 μm in height at the end of macro-scale applicators, a transfer molding process was employed. The fabricated microneedle pens were then applied to mouse corneas. The microneedle pens successfully delivered rhodamine dye deep enough to reach the stromal layer of the cornea with small entry only about 1000 μm^2. When compared with syringes or 30G needle tips, microneedle pens could achieve more localized and minimally invasive delivery without any chances of perforation. To investigate the efficacy of microneedle pens as a way of drug delivery, sunitinib malate proven to inhibit in vitro angiogenesis, was delivered to suture-induced angiogenesis model. When compared with delivery by a 30G needle tip dipped with sunitinib malate, only delivery by microneedle pens could effectively inhibit corneal neovascularization in vivo. Microneedle pens could effectively deliver drugs to thin tissues without impairing merits of using microneedles: localized and minimally invasive delivery.

1. Introduction

Microneedles (MNs) have been rapidly developed in the field of drug delivery. MN insertion into target tissues creates temporary micro pathways in biological tissues with minimal damage. Drug transport is enhanced through the micro paths and results in enhanced efficacy [1]. MNs are manually applied in a patch form to the back of animals where the backbone supports the dorsal skin [2,3] or to the lobe of the ear [4] for easier insertion of MNs. To improve the manual application of MNs, various MN applicators were previously developed including roller discs with protruding MNs [5], MN patches with impact spring [6], elastic-band type wrapped around the human arm [7], flexible sheet type [8], pen-type hollow MNs [9] and insertion of MN patches in vibrating environment [10]. However, MN treatment often fails due to uncontrolled insertion of MNs when target tissue has inherent elasticity or irregular surfaces [11]. To ensure their reproducible penetration into the desired depth, uniform and consistent external force needs to be applied to MNs. However, when MNs are inserted manually, it is difficult to exert uniform and reproducible forces on target tissues.

Recent development of ocular MN devices demonstrated that the efficiency of drug delivery to the inside of the eye could be enhanced with the aid of MNs [12,13]. However, application of MNs to ocular tissues such as the cornea or sclera is extremely challenging due to the round surface and lack of a supporting pressure that is required for reliable MN insertion. In particular, when an in vivo MN study is conducted with small animals such as mice or rats, application of MNs to a target location on the ocular tissue, whose size is less than a few millimeters, becomes a major obstacle. The above-mentioned conventional patch, roller, band, or sheet-shaped MNs are inapplicable to such small and curved ocular tissues. Thus, it is critical to have a MN design and application system suitable for ocular tissue application for small animals.

Here we propose a "transfer-molding" technology that enables repetitive fabrication of single or an array of MNs on the tip surface of a small rod that can be inserted into a small target region of ocular tissue (Fig. 1). To enhance the reliability of MN insertion, a spring-loaded MN applicator system, MN pen (MNP), was also custom-fabricated. Use of this MNP allows for easier handling of the MN by healthcare professionals and the spring force enables impact insertion of MN into a target tissue with minimal damage to MN tips. Drugs were coated on the surface of MN tip using dip-coating method [12,14].

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http://dx.doi.org/10.1016/j.jconrel.2015.04.041
0168-3659/

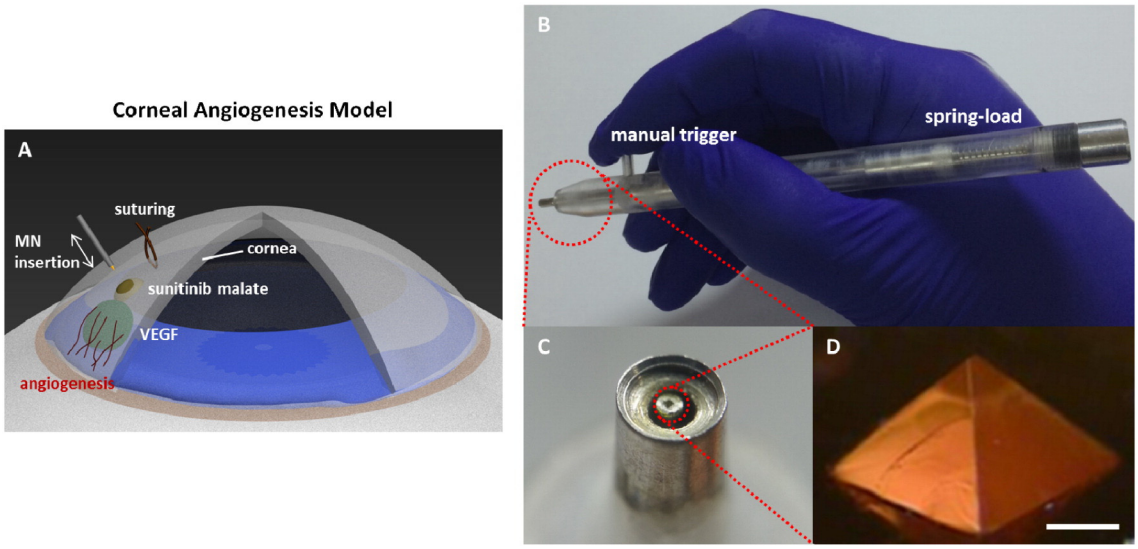

Fig. 1. (A) Schematic diagram of cornea angiogenesis model. (B) Photograph of spring-loaded microneedle pen (MNP). (C) MN guiding structure at the end of MNP. (D) Transfer molded MN structure on the tip end of MNP. Scale bar: 100 μm.

To evaluate the efficacy of the MNP system, mouse cornea was used as a target tissue. Mouse cornea is a small and thin tissue with a diameter of 2.6 mm and a thickness ranging from 68 to 137 μm and it is weakly supported by aqueous humor below [15]. The efficacy of MNP treatment was assessed using suture-induced corneal angiogenesis models. Using a rhodamine B dye (RB) as a model drug, the degree of drug distribution was measured immediately after the MNP application and the remaining edematous change was also recorded one day after the MNP application. After the inhibitory function of sunitinib malate (SM) against VEGF-induced angiogenesis confirmed in an in vitro study, SM-loaded MNP systems were prepared. The SM-MNP was applied to the suture-induced corneal angiogenesis model and inhibition of the corneal neovascularization was confirmed.

2. Materials and methods

2.1. MNP fabrication

MNP was fabricated by chemical etching of silicon wafer to create MN female mold and subsequent transfer molding to attach MN to the tip of a spring-loaded applicator. <100> oriented and 100 nm thick silicon nitride (Si_3N_4) deposited wafer was used as a starting material. A layer of photoresist (SU-8, MicroChem, USA) was deposited onto the Si_3N_4 layer as an etch mask. A lithographic film mask (Nanosys, South Korea) containing a square array pattern (200 × 200 μm^2 with 500 μm spacing), was positioned on top of the photoresist (PR) layer. The wafer and PR were then exposed to ultraviolet (UV) light through the mask using an optical mask aligner. The exposed PR was removed by soaking the wafers in a liquid developer leaving the desired PR pattern on the Si_3N_4 layer. Then the Si_3N_4 layer was also etched to form cavity patterns using a reactive ion etching process. Subsequently, the wafers were dipped into a KOH etchant (45% KOH in DI water, 90 °C, 100 min) for anisotropic etching to form deeper pyramid shaped micro cavity array (A/R = 0.7, 140 μm of depth) on the silicon wafer [16,17]. An SU-8 male MN array mold was prepared by casting SU-8 on the etched silicon mold and additional casting of PDMS on the SU-8 mold created PDMS female MN molds.

MN structure was fabricated on the tip of a macro scale applicator using a "transfer molding" technique (Fig. 2). SU-8 resin was deposited on a PDMS mold containing female MN cavities and placed in a vacuum chamber for 2 min to remove air trapped in the MN cavities. SU-8 residue on the mold was cleaned by doctor blading. The SU-8 filled in the MN female cavities was soft-baked in 90 °C for 4 min. The tip of an MN applicator was dipped into SU-8 resin and lifted up to form a SU-8 droplet on the surface (Fig. 3A). The applicator tip was moved close to one of the MN cavities filled with SU-8 and gently lowered down until it makes a contact to the surface of the PDMS cavity (Fig. 3B). With the contact maintained, both the SU-8 droplet and SU-8 filling the MN cavity were exposed to UV for 4 min (Fig. 3C). Lifting of the applicator de-molded the cured MN-shaped SU-8 connected to the applicator tip (Fig. 3D).

2.2. Drug loading on MN

A mixture of drug and carrier materials was prepared and loaded on the surface of MN tips using a dip coating method (Fig. 4). Rhodamine B (R6626, Sigma Aldrich, USA), Evans blue (E2129, Sigma Aldrich, USA), or sunitinib malate (S8803, LC Laboratories, USA) were used as model drugs in this experiment. The model drugs were thoroughly mixed with a polymer solution of carboxymethyl cellulose sodium salt (CMC) (C5013, Sigma Aldrich, USA) or methyl cellulose (MC) (94378, Sigma Aldrich, USA) as a viscosity enhancer in DI water or dimethyl sulfoxide (DMSO) (Samchun, South Korea) as a solvent of model drugs to form the final formulation for dip coating. The mixing ratio of DI water, CMC and rhodamine B (RB) or Evans blue (EB) in the formulation was maintained at 25:1:5 (weight ratio) for demonstration of controlling MN insertion site in ocular tissue of mouse. The mixing ratio of DMSO, MC and sunitinib malate (SM) was maintained at 25:1:1 (weight ratio) for treating angiogenesis in mouse eye in vivo. After dip coating, the solvent such as DI water or DMSO was evaporated by air drying for 4 h. The amount of SM coated on the MN surface was measured using a microplate reader (SpectraMax M5, Molecular Devices, USA) after its dissolution in DMSO. Under the excitation and emission wavelengths centered at 475 and 535 nm, respectively, the fluorescence of the dissolved solution was compared with standard curves. The amount of SM coated on the MNs was estimated to be 100 ± 11 ng per MN.

2.3. MN insertion into the cornea

C57BL/6J mice purchased from Central Lab. Animal Inc. (South Korea) were used in this study. All animal experiments were approved by the Institutional Animal Care and Use Committee of Seoul National University and were in strict agreement with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. 8-week-old C57BL/6J mice underwent general anesthesia induced by zoletil 50 (15 mg/kg) and lumpun (5 mg/kg) and topical anesthesia induced by proparacaine eye drop solution (0.5%; Alcon Inc., South Korea). MNs