Fabrication of Swellable Microneedle using Methacrylated Hyaluronic Acid for Extraction of Biomarkers in Ocular Fluids

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Introduction: Interstitial fluid (ISF) in the ocular tissue has many biomarkers from ions to proteins. Since the concentration of biomarkers such as lactate and cytokine increase in the presence of cancer, monitoring the level of biomarkers is important to diagnose and treat the diseases. However, there are limited number of tools to collect disease markers from tissue although there are many analytical methods. Herein this study, we developed swellable microneedle (MN) device which was minimally invasive and pain-free to detect the levels of biomarkers from ISF. First, methacrylated hyaluronic acid (MeHA) were synthesized and we fabricated swellable MeHA MNs by a combination of photo-crosslinking and transfer molding. Then, the mechanical stability was confirmed by *ex vivo* compression tests and *in vitro* extraction of model biomarkers from phantom tissue was demonstrated.

Methods: Hyaluronic acid (HA) is the natural polysaccharide consisting of nonsulfated glycosaminoglycan. To synthesized MeHA which is photo-crosslinkable with UV curing to swell without dissolving [1], methacrylation of HA is performed by mixing dimethyl formamide (DMF), methacrylic anhydride (MA) and sodium chloride (NaCl) in a 2% HA solution while mixture maintained at pH 8~9. After reaction with ethanol, the precipitation was dialyzed for a week and lyophilized for stable storage. MeHA MNs were fabricated by transfer molding. A silicon mater mold was replicated to form poly(dimethylsiloxane) (PDMS) mold. A 5% MeHA solution was filled in the MN cavity of the PDMS mold. After drying, a substrate of the MN array was removed by doctor blading and the molded MN within the cavities was lifted off by the tip of an applicator with transfer molding (**Figure.1**). To confirm whether the MeHA microneedles could penetrate the scleral tissue and extract ISF, compressive mechanical test was performed with a universal testing machine (UTM). The tissue penetration forces which was measured by insertion of rigid glass MN (tip diameter: 9 μm) compared with the reactive force of MeHA MN during compression [2]. For *in vitro* extraction tests, MeHA MN was inserted into the 10 % gelatin phantom tissue and maintained for 10 min. The gelatin tissue phantoms contained 1mM of rhodamine B (RB, Mw: 480 Da) and FITC-dextran 4K (FD4, Mw: 4 kDa) which was similar concentration of protein in the ISF. After extraction, molecules were released by centrifuged at 10,000 rpm for 10 minutes. The concentrations of the extracted molecules were measured by a plate reader.

Results: The MeHA which was crosslinked by 3.060 J/cm^2 of UV exposure swelled after 5 sec of immersion in deionized water (DIW) and its swelling ratio was 305.5 ± 17.8%. A single MeHA MN had 640 μm of height, 400 μm of base width and 10.97 ± 1.45 μm of tip diameters (**Figure. 2(a)**). After swelling, the single MeHA MN maintained its structure without any disintegration of the structure (**Figure. 2(b)**). The extracted concentrations of RB and FD4 were 107.84 and 0.83 ng/ml, respectively (**Figure. 2(c)**). These extracted amounts of the model biomarkers were large enough for measurement by ELISA or mass spectrometry. The extraction efficiency depended on the molecular weights of the model biomarkers. The penetration forces of the porcine scleral tissues with glass MN insertion were defined as the first peak of the force vs. displacement curves [2]. While the penetration force of the sclera was 0.026 N, the MeHA MN did not fail even above the penetration force of the sclera (**Figure. 3**).

Conclusions: In this study, MeHA was successively synthesized and single MeHA MN was developed by transfer molding. Crosslinked MeHA MN had sufficient strength for puncture and penetration of scleral tissue as well as extraction efficiency.

References:

[1] H Chang. Adv. Mater. 2017; 29, 1702243.
[2] SH Park. Acta Biomater. 2016; 44, 286-294.

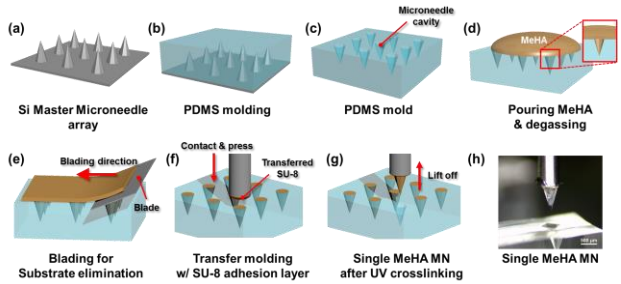

Figure 1. Fabrication process of single MeHA MN by transfer molding.

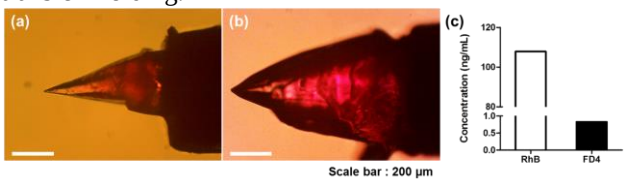

Figure 2. (a) Before and (b) after swelling of MeHA MN, (c) concentrations of extracted molecules

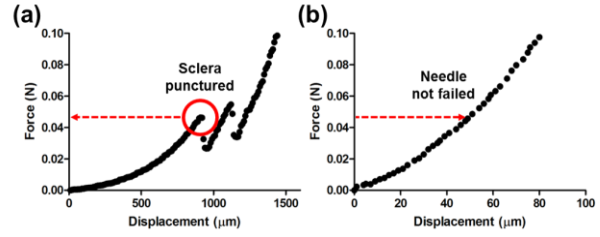

Figure 3. Force vs. displacement curves (a) by insertion scleral tissues with glass MN and (b) by compression of single MeHA MN