**Supporting Information**

# Comparative Study of Dissolving Hyaluronic Acid Microneedles with Trehalose and Poly(vinyl pyrrolidone) for Efficient Peptide Drug Delivery

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## MATERIAL AND METHODS

### Dissolving microneedle fabrication via DAB

Sodium hyaluronate (HA, intrinsic viscosity ([η]) = from 0.1 to 1.0 $m^3$/kg, Bloomage Freda Biopharm., China) was used as matrix materials to fabricate dissolving microneedles via a novel droplet-born air blowing (DAB) method. Both trehalose (Ashland Inc., KY, USA) and poly(vinyl pyrrolidone) (PVP, molecular weight 10 kDa, Ashland Inc., KY, USA) solutions were prepared in distilled water (DW) at the concentration of 5% w/v. HA powder was added to the trehalose or PVP solutions (HA concentration = 13.2% w/w). Next human parathyroid hormone (1-34) (hPTH) powder was added to the polymer solutions and the mixture was stirred using a planetary vacuum mixer.

Microneedle patches were prepared by solution dispenser (ML-5000X, Musashi, Japan) and automated X, Y and Z-stage (Nexgen, CA, USA). HA solution in DW (13.2% w/w) was used for base layer. After drying the base layer overnight at room temperature, the HA solution containing hPTH with or without trehalose or PVP were dispensed onto two base arrays of upper and lower plates. Dispensed viscous solutions on the plate were contacted with each other and elongated. After that, symmetric air was applied through elongated gap to dry. Finally, two plates were separated to isolate microneedle patches.

### Characterization of dissolving microneedle

After fixing the microneedle patch on a rigid stage so that the floor and the microneedles were parallel, the vertical length and the tip thickness of the completely dried microneedle patch were measured

using an optical microscope (Leica, Wetzlar, Germany). Vertical displacement-force of single microneedle was measured by a displacement-force test machine (Z0.5TN, Zwick/Roell, Ulm, Germany). After fixing the microneedle patch on a rigid stage, the fracture force of single microneedle was measured by moving the probe vertically at a speed of 1.1 mm/s. The displacement-force test machine recorded the axial force required to move the probe as a function of distance. The maximum force before immediate force drop was recorded as fracture force of single microneedle.

***In vitro* releasing study**

To evaluate of the dissolution profile of microneedle patches, gelatin hydrogel was used. Briefly describing the manufacturing process of gelatin hydrogel, 6.5 g of gelatin was added to 10 mL of DW and hydrated for 30 min. After this, the gelatin solution was heated upto 60 ℃ in the water bath and gently stirred until the gelatin was completely dissolved. This solution was poured into a petri dish, after then water was evaporated at room temperature overnight until the moisture content reaches 35% to 40%. HA, HA/trehalose, and HA/PVP microneedle patches containing a random peptide (33 amino acids), exhibiting a similar molecular weight to hPTH (34 amino acids), were prepared according to the protocol of hPTH-loaded MNs described above. MNs were inserted into the gelatin hydrogel using applicator. At 0, 10, 30 and 60 min after insertion of the microneedle patches, the patches were detached from gelatin hydrogels. For the dissolution of remaining patches, MN patches after insertion were sufficiently immersed in the dilution buffer (acetonitrile (ACN): 0.2 M sulfate buffer = 25:75) and dissolved for 1 h with gentle stirring. After that, the

peptides were quantified using high performance liquid chromatography (HPLC) system with 215UV detector and Empower software was used for RP-HPLC method (Alliance, Waters, MA, USA). For the reverse phase HPLC, the reversed-phase C18 column (octadecylsilyl silica gel 150 mm X 4.6 mm, 3.5 μm and 300Å) was used. Sulfate buffer at 0.2 M concentration was prepared with 28.4 g/L of sodium sulfate and its pH was adjusted to 2.3 with $H_3PO_4$. The mobile phase A consists of ACN : 0.2 M sulfate buffer (10:90) and the mobile phase B consists of ACN : 0.2 M sulfate buffer (50:50). The gradient program was as follow: 0-35% mobile phase B in 5 min, 35-40% mobile phase B in 30 min, and 40-100% mobile phase B in 10 min. Flow rate was 1.0 mL/min. Remaining peptide amount (%) was calculated in the comparison of the total amount of peptide in the patch, which was set to 100%.

**Animal study of PTH loaded microneedle patch**

Methods were approved by the Institutional Animal Care and Use Committee at the Korea conformity laboratory (IA18-00023). Animal study was designed and conducted under the Korean Ministry of Food and Drug Safety (KMFDS) notice no. 2015-83 'Test standards for pharmaceuticals'. One day before the administration of the microneedle patch, the hair of the back skin of the Sprague Dawley rats (SD rats, Orient Bio Inc., Seongnam, South Korea) was cleanly removed using a hair removal cream (Veet, Reckitt Benckiser, Berkshire, UK). Microneedle patches were applied to each 8-week-old SD rats using a punch type applicator manufactured by Raphas (Seoul, South Korea), and 20 or 60 μg of hPTH was administered per animal. Using a sterilized tape, the patches were fixed on the body of rats. Four hundred

fifty μL of blood samples were collected from jugular vein of rats at 0 min, 5 min, 15 min, 30 min, 1 h, 2 h, and 4 h after administration for formulations A-C and 3 min, 6 min, 10 min, 15 min, 25 min, 50 min, 2 h, and 3.5 h after administration for formulation D.

The plasma concentration of hPTH was quantified using a High Sensitivity Human PTH (1-34) ELISA Kit (Quidel Corp., San Diego, CA) according to the instructions supplied by manufacturer. Pharmacokinetic parameters were calculated using a standard non-compartmental model (WinNonlin Phoenix, Pharsigt corp. Mountain view, CA, USA).

**SUPPLEMENTARY FIGURES**

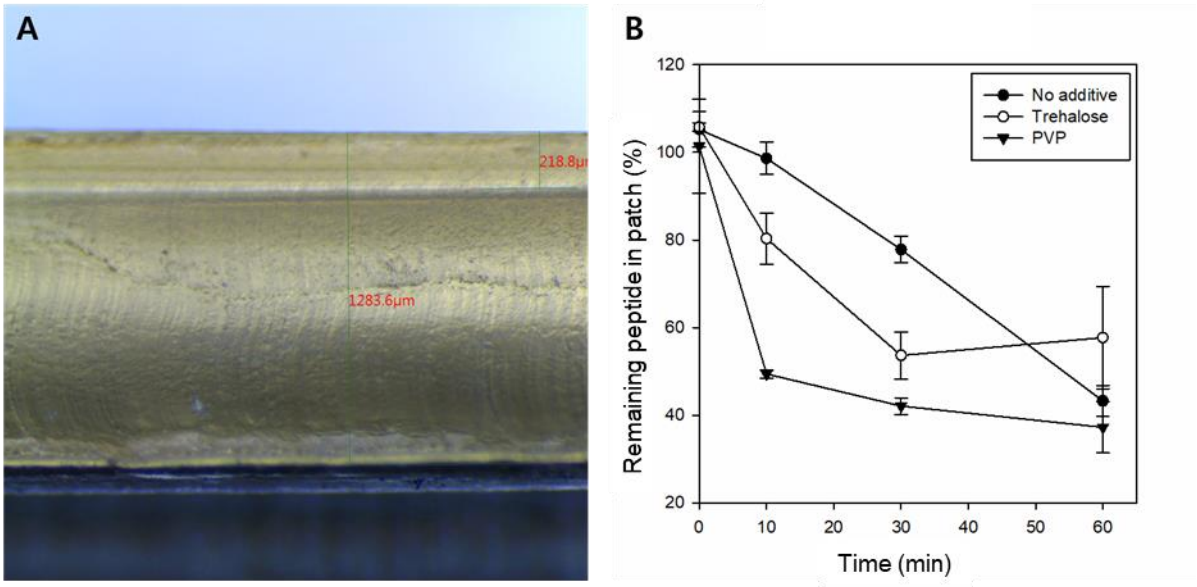


**Figure S1.** In vitro drug releasing test with HA, HA/trehalose, and HA/PVP microneedle patches using gelatin hydrogels. (A) Cross-section of the gelatin hydrogel as an in vitro skin model for the evaluation of microneedle performance. (B) Release profiles of random peptides, exhibiting a similar molecular weight to hPTH, from different microneedle patches to gelatin hydrogels. Closed circle: HA microneedle, open circle: HA/trehalose microneedle, closed inverted triangle: HA/PVP microneedle. At different time points after microneedle insertion, microneedle patches were withdrawn and dissolved in the dilution buffer (acetonitrile : 0.2 M sulfate buffer = 25:75, see the material and method section) to measure the amount of remaining peptide in patches.