Electronic Supplementary Material (ESI) for Biomaterials Science.


*Supporting Information for*

# Double-Layered PLGA/HA Microneedle Systems as a Long-Acting Formulation of Polyphenols for Effective and Long-Term Management of Atopic Dermatitis

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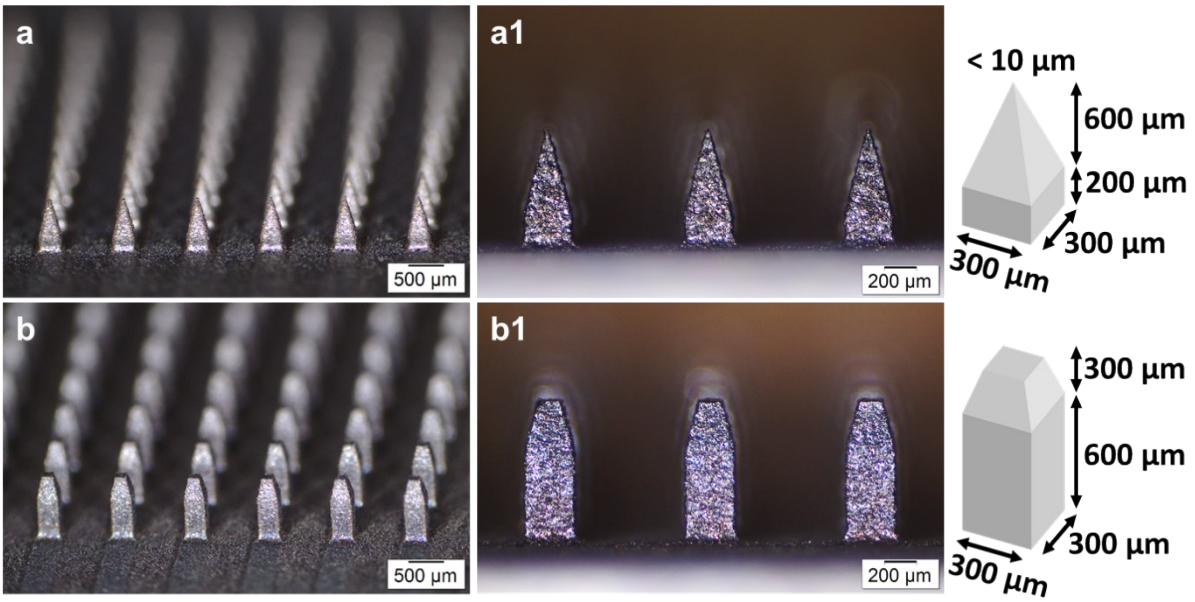


**Figure S1**. Bright-field micrographs of the metallic master structures used for preparing the CUR-loaded PLGA tip (a and a1) and the GA-loaded HA supporting patch (b and b1). The insets show the detailed specifications of each metallic structure.

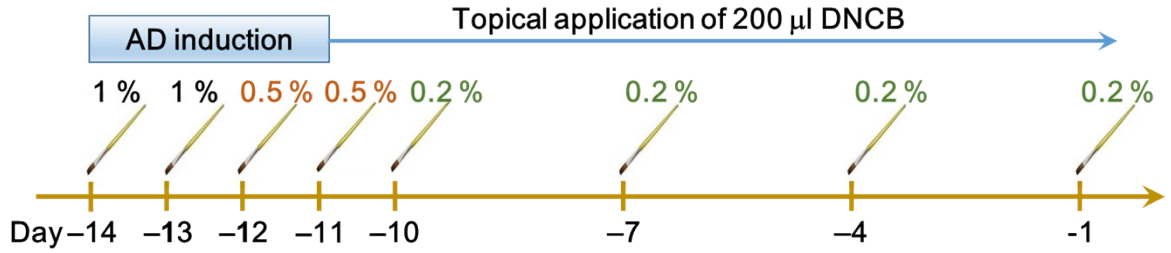


**Figure S2**. Detailed AD induction protocol for Nc/Nga mice.

**Transepidermal Water Loss (TEWL) Measurement**

To measure the integrity of the skin barrier, TEWL was measured using a Delfin VapoMeter® (Delfin Technologies Ltd., Kuopio, Finland). TEWL values were recorded before and after MN insertion until they returned to baseline values, which indicated that the skin was sealed. The control mice underwent only shaving of back hair.

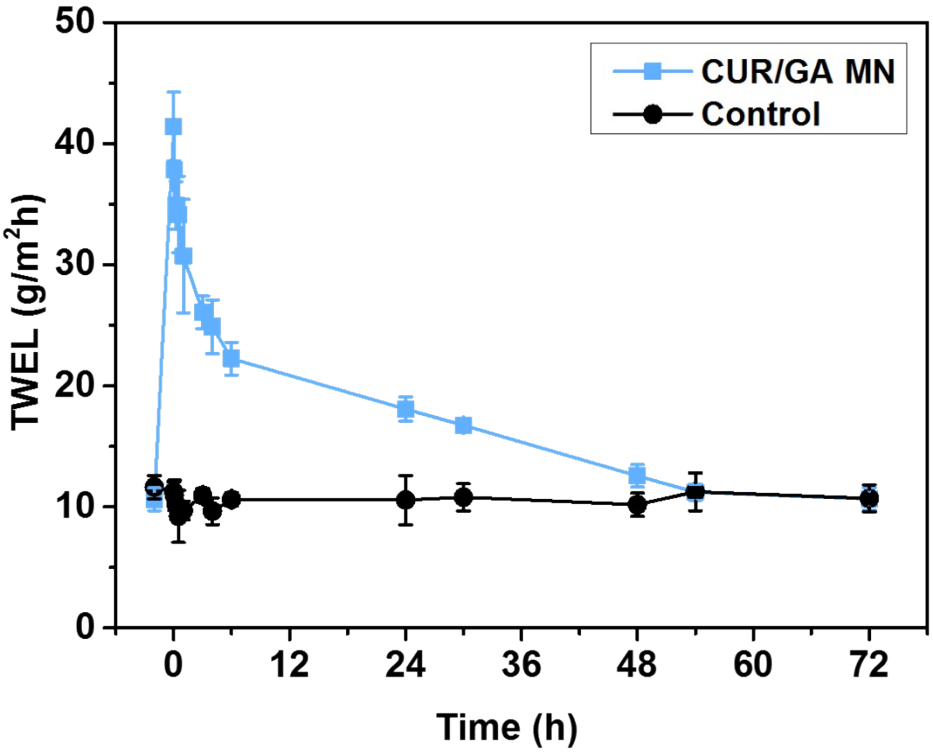


**Figure S3**. Transepidermal water loss (TEWL) from the mouse skin after MN application (n = 5 mice/group): the intact skin without MN insertion (Control); the skin treated with CUR/GA MNs (MN). Data are presented as mean ± SD.

**Determination of Antioxidant Activity of Polyphenols in MN**

The free radical–scavenging capacity of polyphenols in MNs was determined using a 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS•+) decolorization assay, which is based on the reduction of ABTS•+ by antioxidants in samples. After storage in dark 25 °C for 4 weeks, the MN samples were dissolved in 1 mL of DI water stirred in the dark for 20 min. The mixture was centrifuged to obtain the supernatant containing GA/AA and the precipitates (i.e. CUR-loaded PLGA tips). The precipitates were dissolved in 1 mL acetone and stirred in the dark for 1 h at 4°C to extract the CUR from the PLGA tips. The supernatant and the solution obtained from the precipitates were used for the ABTS•+ assay, respectively.

To prepare the ABTS•+ solution, the ABTS aqueous solution (7 mM) was mixed with a 2.45 mM potassium persulfate aqueous solution in equal quantities, and the mixture was allowed to react at RT in the dark for 16 h before use. Then the ABTS+• solution was diluted in DI water an absorbance of 0.7 ± 0.02 at 734 nm. After the addition of 0.1 mL of sample solutions to 2.7 mL of the ABTS•+ solution and stirring in the dark at RT for 10 min, absorbance was measured at 734 nm. The percentage of free radical–scavenging activity of GA/AA in HA layers was calculated using Equation (1):

$$\%\ \text{Free radical–scavenging activity of GA/AA} = [\frac{(A_{control} - A_{sample})}{A_{control}}] \times 100 \quad (1)$$

where $A_{control}$ and $A_{sample}$ are the absorbance of the control and sample, respectively. The ABTS•+ solution was directly mixed with DI water to prepare the control for the GA/AA group.

The percentage of free radical–scavenging activity of CUR was calculated using Equation (2):

$$\%\ \text{Free radical–scavenging activity of CUR} = [\frac{A_{control} - (A_{sample} - A_{background})}{A_{control}}] \times 100 \quad (2)$$

where $A_{control}$, $A_{sample}$, and $A_{background}$ are the absorbance of the control, sample, and background, respectively. For the CUR group, the 3 mL ABTS•+ solution was directly mixed

with 0.1 mL acetone to prepare the control. The 3 mL DI water was directly mixed with 0.1 mL sample solutions to prepare the background.

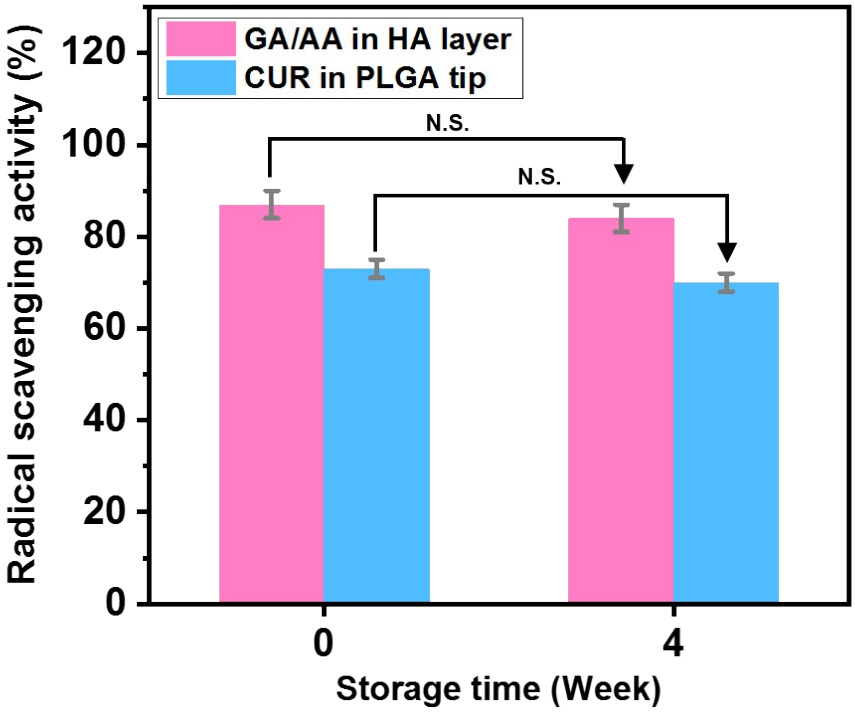


**Figure S4**. The free radical–scavenging activity of GA/AA in HA layer and CUR in PLGA tip before (0 week) and after storage at 25 °C for 4 weeks. Data are presented as the mean ± SD (n = 5). N.S.: no significant difference.

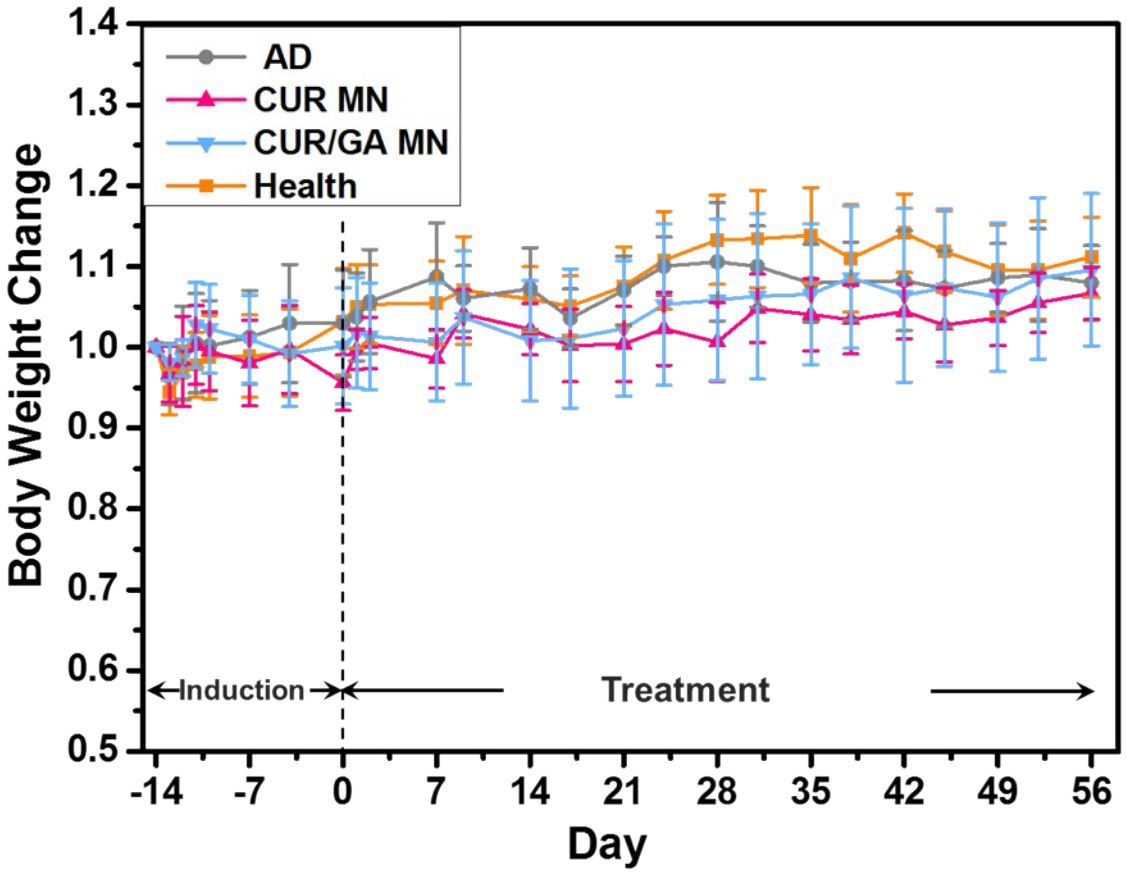


**Figure S5**. Body weight changes of Nc/Nga mice during induction and MN treatment periods. Data are presented as the mean ± SD (n = 5–6 mice/group).