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Hyaluronic Acid Dissolving Microneedle Patch Loaded with Rice Fermentation Filtrate for Improved Moisturizing and Anti-wrinkle Effect

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Introduction:

Not all functional molecules in skin care products can be smoothly absorbed by skins owing to skin barriers including physical barrier, pigment barrier, nerve barrier and immune barrier. The dense keratinocytes are the most important compose of skin physical barrier, which is also the biggest barrier for cosmetic molecules to effectively transmit through the skin. The main methods to promote transdermal absorption of household cosmetic products include chemical methods, physical methods, and pharmaceutical methods. However, they all have some disadvantages on human skins, including bleeding and infection. Therefore, it is urgent to develop a new, efficient and safe household cosmetic delivery system.

Microneedles (MNs) is a new type of physical penetration promoting technology, which is composed of several MNs connected on the base in the form of array. The length of the MNs is 25-1 000 μ m, size and shape can be designed individually according to the needs of treatment.[1] As a new molecules' delivery agent, MNs can break through the cuticle barrier and form multiple micron channels on the skin surface temporarily. The micropores formed on the skin surface by MNs can promote the effective penetration of active ingredients into the skin, so as to achieve the effects of reducing wrinkles, treating scars and stretch marks, whitening the skin and reducing color spots.[2]

HA is the main component of connective tissue, such as human interstitial. Its aqueous solution has significant viscoelasticity, thus it can help to maintain the skin elasticity and fullness. And it also has a strong water retaining effect. Thus, as natural ingredient of human body, HA has both excellent biocompatibility and multifunctional skin care capability, which makes it perfect to be as both needle base material and functional skin care component in dissolvable MNs.

Materials & Methods:

Preparation of MN patches
The HA dissolving MN patches were fabricated by a laser-ablation and micro-molding process. HA aqueous solution (250 mg/mL) mixed with RFE and DDBD was poured over the molds, followed by vacuum treatment (~ 0.08 MPa) to fabricate RFE and DDBD loaded MNs. Then, the samples were dried in a sealed desiccator overnight at room temperature. After being peeled off from the molds, all the final MN patches were sealed up, stored in the desiccators at room temperature and away from light.

Characterization, dissolution progress, and transdermal depth of MN patches
To exam the dissolution progress of MN patches, briefly, MN patches were firmly pressed onto the exterior surface of the skin for 3, 5, 10, 20 min. Then after removing the patch by the exact time, MN patches were observed by a microscope (Olympus, Tokyo, Japan). The freshly excised full-thickness porcine cadaver skin was placed on a microscope slide and then a rhodamine 6G-loaded HA MN patch was skin was investigated by CLSM. Fluorescence signal was detected at the excitation wavelength of 526 nm. After determining the xy-plane with maximum and minimum fluorescence intensity in CLSM, images were obtained from xy-plane at a scan interval of 10 μm in z-axis to observe the dye diffusion in the skin.

In vitro biosafety of HAMNs
HaCaT cells were seeded in a 96-well plate at a density of 10000 cells/well and cultured in Dulbecco's Modified Eagles Medium (DMEM; Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; CellMax, China) and 1% (v/v) penicillin-streptomycin solution. The plate was incubated overnight at 37 ° C in 5% CO2 to allow the cells to attach. Before the experiment, an equal number of the unloaded and RFE and DDBD loaded MNs MN patches were dissolved in equal volume of fresh culture medium, respectively. After 48 h of incubation, all culture medium was removed, and the plate was washed twice with PBS. Subsequently, fresh media and 10% (v/v) Cell Counting Kit-8 (CCK-8; Dojindo, Japan) were added into the plate. The absorbance of each well was measured at 450 nm using an ELISA microplate reader (TECAN Infinite F50, Switzerland) 2 h later.

Transepidermal Water Loss (TEWL) measurement and cuticle moisture content
TEWL is measured by the vapor. The lower the measurement value, the better the skin moisture retention. Moisturemeter is used to measure the cuticle moisture content. The higher the measurement value, the higher the moisture content in the stratum corneum.

Results & Discussion:

Characterization of HAMNs
The dermoscopy images of final MN patches were shown in Figure 1a. Each MN patch consisted of 1800 needles on a baseplate with a height of 330 ± 19 μm, base width of 220 ± 7 μm, and center space of adjacent needles of 500 ± 12 μm. The MNs exhibited well conical shaped with uniform dimension and sharp tips, which was necessary to insert into the skin. The appearance of MN patch is like arc shape to match periocular (Figure 1b). Hydrogel (Figure 1c) was used to help fix the MN patch on the periocular area and provide water to accelerate the dissolution of MNs.

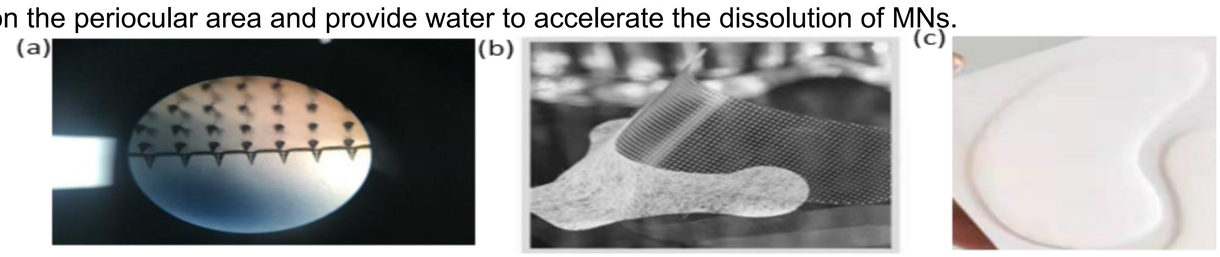

Figure 1. The micro characterizes (a) and appearance of MN patches (b) and the appearance of the assistant hydrogel (c)

Dissolution progress of HAMNs
To examine the vitro dissolution progress of HAMNs, MN patches were firmly pressed onto the exterior surface of the skin for 3, 5, 10, 20 min. After being firmly pressed on the skin, the MNs were fixed onto the using area with the help of assistant hydrogel. As is shown in Figure 2, MNs dissolved smoothly by the time, to balance the penetration depth and using feeling. At the beginning of the use, by pressing the MN patches, a limp and numb feeling was experienced. After 3 minutes, MNs began to dissolve and soften, and the numbness gradually decreased until it disappeared. After 5 to 10 minutes, the dissolution rate of the microneedles is different according to the water supplement rate. After 10 minutes of use, MNs dissolved basically, the tingling sensation disappeared completely, and the active ingredients dissolved and released at the same time. After using for more than 20 minutes, MNs are completely dissolved, the active ingredients are completely dissolved through the microneedles, and the anti-wrinkle polypeptide ingredients are continuously released through the microchannels opened by the microneedles.

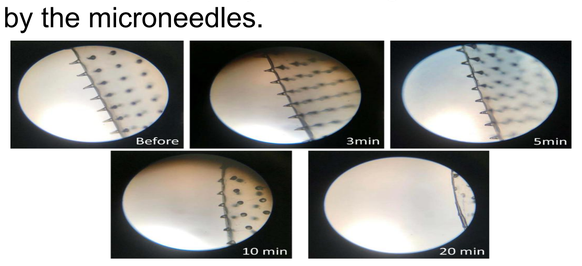

Figure 2. Dissolution progress of HAMNs

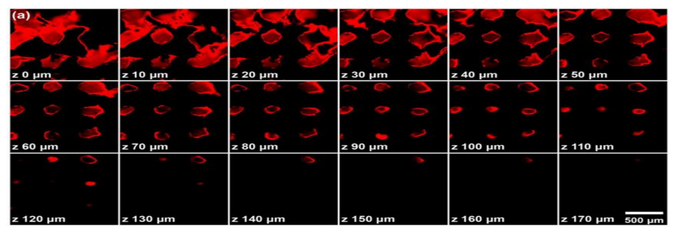

Figure 3. *In vitro* skin penetration characterization of the MNs

Transdermal depth of MN patches
The MN patch was inserted into the full-thickness porcine cadaver skin and kept for 20 min before the removal. The red fluorescence signal of rhodamine 6G was observed by CLSM and the images at varied depths were collected in Figure 3. Clearly, the depth of rhodamine 6G diffusion in the skin was deeper than 150 μm, which achieved comparable or superior performance to previous reports.

In vivo moisturizing and anti-wrinkle effect of RFE and DDBD-loaded HAMNs
As shown Figure 4, the average moisture content of the skin was 53.7% before using the microneedle. After using the microneedle for 2 hours, the moisture content of the skin increased to 63.4%. After using the microneedle for 24 hours, the moisture content of the skin was 70.3%. There was no similar fluctuation in the control group. As shown in Figure 5, it can be that the wrinkles in the corners of the eyes were significantly reduced after using the microneedles for 2 hours.

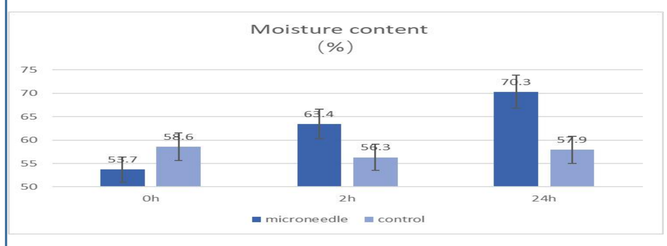

Figure 4. *In vivo* moisturizing effect after using RFE and DDBD-loaded HAMNs

Figure 8. *In vivo* anti-wrinkle effect after using RFE and DDBD-loaded HAMNs

Conclusions:

The dissolving HA, RFE and DDBD showed no significant cytotoxicity to HaCaT cells. The red blood of the skin did not change significantly after 2 hours-using the microneedle. It shows that the safety of microneedles for skin is very high. The higher the TEWL value, the more water lost through the skin, and the worse the barrier function of the stratum corneum. The microneedles only opened the microchannels for a short time, which increased the TEWL, and soon the skin will repair itself. The average moisture content of the skin was 53.7% before using the microneedle. After using the microneedle 24 hours, the moisture content of the skin was increased from 53.7% to 70.3%. Showed in VISIA picture, Microneedles can help to open the skin channel, let the effective ingredients into the skin, the effect is better and faster than the traditional skin care products.

References:

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[2]Prausnitz M (2004), Microneedles for transdermal drug delivery. Adv. Drug Deliv. Rev. 56 (5) 581–587.