A novel transcutaneous vaccine formulation using a self-dissolving microneedle patch

Sachiko Hirobe¹, Kazuhiko Matsuo¹, Ying-Shu Quan², Fumio Kamiyama², Naoki Okada¹, Shinsaku Nakagawa¹

¹Laboratory of Biotechnology and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, 565-8071, Japan; ²CosMED Pharmaceutical Co. Ltd., Kyoto, Kyoto, 601-8014, Japan sachi-be@phs.osaka-u.ac.jp

ABSTRACT SUMMARY
We have developed a dissolving microneedle patch (MicroHyala; MH) for use as a device of transcutaneous vaccination. In this study, we demonstrate that MH delivered the antigen included in the microneedles intradermally safely and surely and was able to elicit the antigen-specific immune response effectively.

INTRODUCTION
Vaccination, which protects us from illness and death by infectious disease, has greatly contributed to improving human health globally. However, injection as major vaccination system is painful, requires medical personnel with technical skill, and comes with the risk of needle-related diseases and injuries. Moreover, antigen solutions require cold chain storage and transportation systems. These disadvantages of conventional injections hamper the spread of vaccination technologies to developing countries. One of the innovative methods that resolve these issues is transcutaneous immunization (TCI) systems (1).
Microneedles can physically penetrate the stratum corneum and directly deliver antigen painlessly into the skin with easy application. Most conventional microneedles suffer from the risk of fracture, which leaves metal, stainless steel, or silicon microneedle fragments in the skin. We have developed the novel microneedles made of hyaluronic acid, which dissolve in the skin. Because our self-dissolving microneedle patch (MicroHyala; MH) leave no bio-hazardous sharp medical waste and remove the risk of secondary infection by used-needles, we believed that MH is extremely promising as a novel TCI device.

In this study, we investigated the characteristics, safety, and efficacy of the transcutaneous vaccine formulation using MH in animal experiments (2-4)

EXPERIMENTAL METHODS
Preparation of MH
The solution of sodium hyaluronate and hydrolyzed collagen was cast into micro-molds and dried in a desiccator at room temperature. Microneedle length varies from 200 μm to 800 μm. In the present study, we used MH800 having 200 microneedles, which is 800 μm length, per a patch (0.8 cm²).

Microneedle dissolution and the antigen delivery of MH800
MH800 was applied to the back skin of mice or rats by using a handheld applicator. After MH800 application for 5 or 60 min in rats, microneedles were immediately observed under stereoscopic microscope for analysis of microneedle dissolution. Additionally, antigen delivery was analyzed using the skin after the application of each MH800 containing fluorescent-labeled ovalbumin or silica particles (diameter: 300 nm).

Assessment of skin irritation by MH800 application
Skin irritation was scored with time according to Draize test for signs of erythema or edema after MH800 application for 30 min.
Tetanus toxoid (TT) and diphtheria toxoid (DT) vaccination
Combined TT and DT-containing (10 μg each) MH800 were applied onto the back skin of hairless rats for 6 h. Control rats were treated with subcutaneous immunization (SCI). These procedures were repeated five times at 2-week intervals. Toxoid-specific IgG titer in sera was measured by ELISA, and its neutralization activity was determined by monitoring survival of mice injected with the mixture of serum and lethal tetanus toxin.
Influenza hemagglutinin (HA) vaccination
HA (0.4 μg) from mouse-adopted influenza virus (A/PR/8/34)-containing MH800 were applied to the shaved back skin of BALB/c for 6 h. As control, mice were treated by either intramuscular immunization (IMI) or intranasal immunization with 10 μg cholera toxin (INIC-T). The TCI (placebo) group received antigen-free MH800. These procedures were repeated twice at 4-week intervals. HA-specific IgG and IgA titer in sera or nasal washes were measured by ELISA, and hemagglutination inhibition (HI) titer was determined by HI test.
Virus challenge experiment
Mice immunized with HA (A/PR/8/34) were challenged by intranasal instillation of 5 × 10⁶ PFU of influenza virus (A/PR/8/34) 2 weeks after the last vaccination. Mice were monitored for weight loss and signs of illness every day. Six days after the challenge, influenza viral load in lungs was determined by plaque assay.

RESULTS AND DISCUSSION
Microneedle dissolution and the antigen delivery of MH800
The half length of microneedles dissolved after application for 5 min, and microneedles fully dissolved by treatment for 60 min in rats (Fig. 1A). In addition, the MH800 could deliver not only soluble antigens but also particulate antigens into the epidermis and the dermis (Fig.
These results indicated that the MH800 delivered various antigens into the skin regardless of the antigen form by application for at least 60 min.

Skin irritation caused by MH800 application

After MH800 application for 30 min, edema was not observed in any rats. Slight or moderate erythema induced by MH800 application gradually disappeared within a few days, indicating that MH800 was a safe device for the skin.

Efficacy of TT and DT vaccination

Rats vaccinated by TCI using MH800 had an increased anti-TT and anti-DT IgG titer after the first vaccination, which was comparable to that of the SCI groups. Additionally, antibody in sera from rats vaccinated by TCI using MH800 neutralized lethal tetanus toxin because mice injected with the mixture of serum and toxin did not die. These results indicated that our TCI system induced toxoid-specific antibody which can neutralize toxin and blocked the appearance of infection disease.

Efficacy of influenza HA vaccination

The results of the anti-HA IgG titer measurement indicate that the IgG production profile in the TCI was similar to that of IMI and INI-CT groups (Fig. 2A). In nasal washes, Anti-HA IgA, which has a critical role in protecting against infection, was little detected in TCI and IMI groups, suggesting that our TCI system could not induce an HA-specific IgA response to the same extent as the conventional injection system. We then analyzed the HI titer of sera as the serological measure of functional antibodies. HI activity of TCI group was comparable to that in the IMI groups. These results indicated that TCI using MH800 induced strong antibody responses with significant HI titers.

Protection against influenza virus challenge

After A/PR/8/34 influenza virus challenge, mice of TCI (placebo) group succumbed; they lost 30% of their body weight and showed worsening of symptoms (Fig. 2B). In contrast, TCI group mice showed no remarkable weight loss or other symptoms of illness, similar to the IMI and INI-CT groups. Moreover, the influenza virus load in the lungs of the all immunized group was below the detection limit, demonstrating that our TCI system provided protection equal to that of IMI and INI-CT. Therefore, our TCI system confers protective immunity as effectively as intramuscular and intranasal administration.

On the basis of these results, we are performing translational studies for clinical application of this TCI system. We confirmed that application of antigen-free MH800 was safe in human, and then clinical studies to assess safety and efficacy of MH800 in the delivery of seasonal trivalent influenza HA antigens are in progress.