European Journal of Pharmaceutics and Biopharmaceutics 119 (2017) 91–95

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

# Enhancement of transdermal protein delivery by photothermal effect of gold nanorods coated on polysaccharide-based hydrogel

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ARTICLE INFO

*Article history:*
Received 10 June 2016
Revised 12 January 2017
Accepted in revised form 5 June 2017
Available online 10 June 2017

*Keywords:*
Controlled release
Gold nanorod
Hydrogel
Near-infrared light
Photothermal effect
Transdermal protein delivery

ABSTRACT

Transdermal protein delivery is a useful and attractive method for protein therapy and dermal vaccination. However, this delivery method is restricted by the low permeability of the stratum corneum. The purpose of this study was to develop a transdermal delivery system for enhancement of protein permeability into the skin. First, we prepared a transparent gel patch made of polysaccharides with gold nanorods on the gel surface and fluorescein isothiocyanate-modified ovalbumin (FITC-OVA) inside. Next, the gel patch was placed on mouse skin to allow contact with the coated gold nanorods, and irradiated by a continuous-wave laser. The laser irradiation heated the gold nanorods and the skin temperature increased to 43 °C, resulting in enhanced translocation of FITC-OVA into the skin. These results confirmed the capability of the transdermal protein delivery system to perforate the stratum corneum and thus facilitate the passage of proteins across the skin.



## 1. Introduction

Protein-based drugs have important roles in the treatment of various diseases, including diabetes, osteoporosis, and cancer. Although protein delivery is mostly being performed by the parenteral route, it has some drawbacks such as the requirement for repeated administrations and low patient compliance [2]. Consequently, transdermal delivery is an attractive method for protein delivery compared with other administrations such as traditional needle injection and oral route. However, it is well known that the outermost layer of the skin, the stratum corneum, is a hydrophobic barrier. This is a hindrance for the delivery of hydrophilic macromolecules such as proteins [6]. For a successful transdermal delivery of hydrophilic macromolecules, many approaches have been investigated, with some of the major techniques being microneedles, thermal ablation, iontophoresis, and photomechanical waves [10]. Thermal ablation is a promising mechanism for increasing the permeability of the stratum corneum. It provides a non-invasive technique to increase the skin permeability through microchannels into the skin. Transdermal protein delivery using thermal ablation can be attained by local heating of the skin surface to vaporize the tissue (i.e. removing the stratum corneum at the sites of heating) [4,8].

Rod-shaped gold nanoparticles, gold nanorods, have unique optical properties. [5,7,13]. They show two absorption bands in the visible and near-infrared (NIR) regions corresponding to transverse and longitudinal surface plasmon oscillations, respectively. The absorbed light energy is efficiently converted into heat, the so-called photothermal effect [3]. We previously prepared a formulation comprising a solid-in-oil S/O) dispersion of ovalbumin OVA) and gold nanorods [9]. The dispersion in a cylinder cup placed on mouse skin was irradiated by NIR light, and efficient transdermal OVA delivery and anti-OVA antibody induction were observed. The photothermal effect of the gold nanorods in the S/O dispersion increased the permeability of the stratum corneum by thermal ablation. With using this technique, we observed that the generated heat affected not only the stratum corneum but also the deeper tissues like the dermis and subcutaneous tissues. It can be expected that heating to the deeper tissues might affect severe damage of the skin.

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http://dx.doi.org/10.1016/j.ejpb.2017.06.005
0939-6411/

In order to perform a successful transdermal delivery, the major challenge is to penetrate only the main skin barrier, the stratum corneum without heating of the deeper tissues. Therefore, we expected that it would be possible to heat only the stratum corneum by direct contact of the gold nanorods onto a localized area of the skin surface and followed with NIR irradiation for photothermal effect. This experiment worked well for protein delivery from an aqueous solution into the skin without using detergents or oil. However, the system still had some weaknesses and the cylinder cup used in this technique was not suitable for clinical application [12].

In the present study, we improved the system, in which a patch system was employed instead of a cylinder cup that might be an obstacle for clinical application. However, in our system, the patch should be transparent for NIR because NIR irradiation is required to enhance transdermal protein delivery. Most of commercially available patches for transdermal drug delivery are not transparent. Therefore, we newly developed a transparent polysaccharide hydrogel coated with gold nanorods and placed on the skin surface to allow contact with the gold nanorods. It was expected that using the transparent gel patch system, the stratum corneum would be heated by the photothermal effect of the gold nanorods during NIR light irradiation, and that protein translocation would be enhanced by thermal ablation of the skin.

## 2. Materials and methods

### 2.1. Materials

Gold nanorods were provided by a joint research project between Mitsubishi Materials Corp. and Dai Nippon Toryo Co. Ltd. The mean size of the nanorods was 46 ± 6 nm in length and 10 ± 1 nm in width. Poly (sodium 4-styrenesulfonate) (PSS) and poly (allylamine hydrochloride) (PAH) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Gellan gum (GG) was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Hyaluronic acid (HA) and chondroitin sulfate (CS) were obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan).

### 2.2. Preparation of PSS-PAH-coated gold nanorods

A cetyltrimethyl ammonium bromide-capped gold nanorod solution (10 mL) was centrifuged at 9000*g* for 15 min and the pellet was resuspended in 5 mL of Millipore water. A PSS solution (5 mL; 2 mg/mL in water) was gently added and mixed overnight to allow complete polymer coating. To remove the excess PSS polymer after coating, the coated gold nanorod solution was centrifuged at 9000*g* for 15 min. After resuspension of the pellet in 5 mL of Millipore water, a PAH solution (5 mL; 2 mg/mL in water) was added and mixed overnight. The coated gold nanorod solution was then centrifuged again as described above, and the pellet was resuspended in 10 mL of Millipore water for further experiments.

### 2.3. Preparation of gel patches

GG was mixed with distilled water at various compositions (1.5%, 2%, and 3% w/v) and stirred at 90 °C. Next, different ratios of anionic polysaccharides (CS and HA) were added to these solutions as shown in the supporting information (Table S1) and mixed well for 5 min. The solutions were placed into molds of 1-mm thickness, and the resulting gels were air-dried at room temperature.

### 2.4. Incorporation of fluorescein isothiocyanate-modified OVA (FITC-OVA) into gel patches

A solution of FITC-OVA was prepared as follows. First, 100 mg of OVA (MW: 45,000 Da; Sigma, St. Louis, MO) was dissolved in 20 mL of 0.2 M phosphate buffer (pH 8.0). Next, 2.6 mg of FITC was dissolved in 100 μL of DMSO and added to the OVA solution. The molar ratio of FITC and OVA was 3:1. The mixture was stirred at 4 °C for 24 h in the dark. The mixture was then sequentially dialyzed against 1% sodium bicarbonate aqueous solution pH 8–9) for 2 days and distilled water for 24 h to remove excess FITC, and lyophilized [12]. The prepared FITC-OVA (2 mg) were dissolved in 40 μL of Millipore water. The solution was poured onto the surface of the dried films (1 cm × 1 cm), left overnight to allow hydration, and then dried again.

### 2.5. Release of FITC-OVA from gel films

The gel films were tested for dissolution of FITC-OVA using a fluorescence spectrometer (FP-6600; JASCO, Tokyo, Japan). The receptor chamber containing phosphate-buffered saline (PBS) was maintained at 33 °C and constantly stirred with a magnetic stirrer bar. The FITC-OVA solution was used as a control and the dissolution rates were represented as percentages. The experiments were performed in triplicate.

### 2.6. Animals

Male ddY mice (Kyudo Co. Ltd., Saga, Japan) were used in all experiments. Briefly, 4–5-week-old mice were maintained in a temperature-controlled chamber (24 °C) on a 12-h/12-h light/dark cycle. They were provided with drinking water and feed *ad libitum*. All animal experiments were carried out in accordance with the Guidelines for Animal Care and Use Committee, Center for Animal Resources and Development (CARD), Kumamoto University.

### 2.7. In vitro experiments for temperature increase and translocation of FITC-OVA into skin

Mice were anesthetized by intraperitoneal injection of pentobarbital (8 mg/mL; 150 μL), and their lower dorsal hair was removed with hair removal cream. The mice were euthanized on day 2 after hair removal, and the treated skin was peeled and then stored at −80 °C until use for *in vitro* experiments.

Prior to *in vitro* experiments, gel patches were prepared, in which FITC-OVA was embedded in the gel and gold nanorods were coated on the gel surface. Gel patches without coated gold nanorods were prepared as negative controls. Briefly, FITC-OVA (2 mg) were dissolved in 40 μL of Millipore water. The solution was poured on the surface of a dried film (1 cm × 1 cm) composed of GG and CS/HA, and the film was hydrated to form a hydrogel. After the gel was dried overnight, 25 μL of PAH-coated PSS-nanorods (concentration of gold nanorods: 1.8 mg/mL) was poured on the surface of the gel film, and the film was hydrated to form a gel again for 1 h.

A Franz diffusion cell with a diffusion area of 0.79 $cm^2$ was used for this study [9,12]. The skin was thawed at room temperature for about 30 min, cut to a suitable size, and mounted on a receptor chamber of the diffusion cell filled with PBS. The gel patches were placed on the dorsal mouse skin and then irradiated at a wavelength of 920 nm at 150 mW for 20 min using a continuous-wave (CW) NIR laser (5-mm beam diameter; Alfright Corporation, Madison, WI). The skin temperature was monitored by an infrared thermograph camera (Advanced Thermo TVS-500EX; NEC/Avio, Tokyo, Japan) during the irradiation period. After 24 h of incubation, the treated skin sections were embedded in OCT compound