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A self-adherent, bullet-shaped microneedle patch for controlled transdermal delivery of insulin

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ABSTRACT

Proteins are important biologic therapeutics used for the treatment of various diseases. However, owing to low bioavailability and poor skin permeability, transdermal delivery of protein therapeutics poses a significant challenge. Here, we present a new approach for transdermal protein delivery using bullet-shaped double-layered microneedle (MN) arrays with water-swellable tips. This design enabled the MNs to mechanically interlock with soft tissues by selective distal swelling after skin insertion. Additionally, prolonged release of loaded proteins by passive diffusion through the swollen tips was obtained. The bullet-shaped MNs provided an optimal geometry for mechanical interlocking, thereby achieving significant adhesion strength (~1.6 N cm^{-2}) with rat skin. By harnessing the MN's reversible swelling/deswelling property, insulin, a model protein drug, was loaded in the swellable tips using a mild drop/dry procedure. The insulin-loaded MN patch released 60% of insulin when immersed in saline over the course of 12 h and approximately 70% of the released insulin appeared to have preserved structural integrity. An *in vivo* pilot study showed a prolonged release of insulin from swellable MN patches, leading to a gradual decrease in blood glucose levels. This self-adherent transdermal MN platform can be applied to a variety of protein drugs requiring sustained release kinetics.

1. Introduction

Proteins have essential roles in biological processes, including catalysing biochemical reactions, mediating cell signalling, and are part of the immune system in the body [1]. With the development of recombinant DNA technology, which allows for the mass production of pure target proteins, protein-based drugs have become attractive therapeutic agents for the treatment of various diseases, such as diabetes and cancers [2,3]. Protein drugs are commonly administered *via* the subcutaneous route to increase bioavailability and avoid enzymatic degradation in the gastrointestinal tract and liver [4]. However, this route typically requires the use of hypodermic needles which is fraught with limitations, including poor patient compliance because of pain during injections and a risk of infection [5,6]. An alternative approach is to deliver proteins across the skin. This cutaneous route could provide a comfortable and on-demand protein delivery in a non-invasive or minimally-invasive manner [2,7]. However, because proteins are large hydrophilic molecules, their passive permeation across the skin is prohibited by the stratum corneum (SC), the outermost barrier of the skin made up of dead keratinocytes [8]. Therefore, many studies have been devoted to achieving enhanced delivery of protein therapeutics across the skin. Several enhancement techniques, including iontophoresis [9], electroporation [10], ultrasound [11], and laser ablation [12], increased the efficiency of protein delivery; however, special equipment was required.

Recently developed microneedle (MN) techniques offer an attractive method for protein drug delivery by creating micro-channels through the skin; these channels assist in the percutaneous transport of drugs. Because MNs are micron-sized, they can be applied to the skin without causing pain and with minimal trauma and reduced infection risk

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[13,14]. Three types of MNs (solid, hollow, and dissolvable MNs) have been studied for transdermal protein drug delivery. Drug-coated solid MNs are advantageous for the rapid delivery of macromolecules including proteins [14–16]. They can quickly deliver the desired dose into skin following insertion while the drug amount that can be coated onto the surface of MNs is limited. Hollow MNs enable the infusion of liquid protein formulations and are designed to be fast-acting by delivering intact protein drugs in a buffered solution into deeper layers of the skin [17,18]. However, problematic issues with hollow MNs include complicated MN fabrication steps and blockage of the outlet by surrounding tissues [19]. Drug-loaded dissolvable MNs are prepared using micromolding [20–22] or dwelling [23] processes with drug/polymer solutions. Because the encapsulated drug is rapidly released from the MNs after contact with interstitial fluid following insertion into the skin, sustained delivery of protein therapeutics in a controlled manner is difficult to achieve. In an attempt to achieve a prolonged release of protein drugs, cross-linked MNs, prepared by chemical or physical crosslinking of hydrophilic polymers, were investigated by several research groups [24,25]. The crosslinked MNs that formed hydrogels after uptake of body fluid following skin penetration offered a transport channel for drugs encapsulated in the MN or incorporated in a drug reservoir attached to the hydrogel-forming MN layer, thereby achieving a sustained release of the drug by transporting through the polymer network. It is worth noting that during the encapsulation process of protein drugs in MNs, chemical crosslinkers have to be selected carefully to avoid impacting the bioactivity of protein, which may decrease due to its highly sensitive tertiary structure [26]. The drug reservoir system is designed to overcome this issue with hydrogel-forming MNs that can effectively deliver the drugs from the reservoir through the swollen network; however the release kinetics of drug might be affected by the hydration level of the hydrogel-forming MN layer. In addition, patch-type MN arrays were developed for protein delivery to increase the payload of the drugs, but the adherence of patches applied to skin can be decreased by sweating (perspiring) or showering. Ideally the attachment of the patch should be maintained during normal daily activities to achieve a consistent long-term drug delivery. The chemical adhesive layers of MN patches may cause skin irritation over prolonged use, a potential limitation which should be overcome for long-term applications of the MN patch [27]. Recently, we developed a double-layered MN adhesive with swellable tips, which was inspired by the ability of endoparasitic worms to attach to the intestinal wall of their hosts through expanding a chamber following penetration [28]. While the swellable MN with a conical geometry achieved a firm adhesion to multiple soft tissues, the MN adhesive occasionally failed to interlock with tissue in a relatively shallow penetration.

In the present study, we report a self-adherent transdermal patch for controlled protein drug delivery. As illustrated in Fig. 1a, the double-layered, bullet-shaped MN with drugs loaded in swellable tip can achieve a mechanical interlocking-mediated adhesion by absorbing body fluids after insertion into skin and provide diffusion channels to release loaded drugs from the interlocked tips. This design represents a geometrical optimisation of an adherent MN system that we previously described by increasing the volume of swellable parts, which are beneficial for mechanical interlocking with tissue following insertion as well as the loading capacity of drugs [28]. Because the loaded drugs would be released following the swelling of the tip by passive diffusion through the swollen hydrogel networks, a prolonged and constant drug release would be achieved, similar to that of hydrogel-based drug delivery systems [29–31]. Our work demonstrated that the MN design was effective to achieve a reliable adherence to animal skin, even in dynamic conditions. By using the reversible swelling/deswelling properties of the swellable MN tips, insulin-loaded MN patches show the potential to regulate blood glucose levels in a preclinical model which could provide a non-invasive treatment of type 1 diabetes.

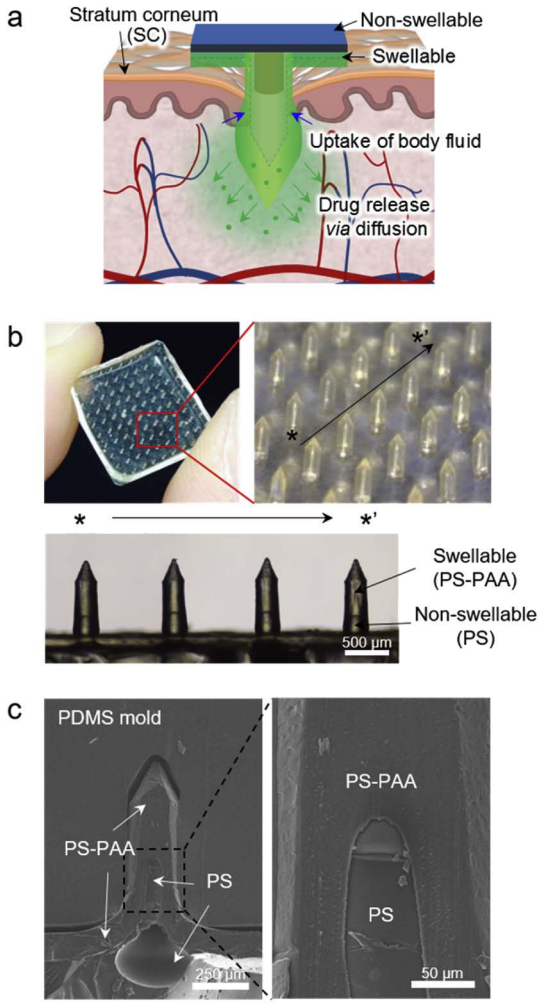

Fig. 1. Concept and images of the double-layered MN patch. (a) Schematic illustration of a water-responsive shape-changeable MN for mechanical interlocking with skin and drug release *via* passive diffusion. (b) Photographs of the bullet-shaped double-layered MN patch with 10 × 10 array in a 1 cm^2. The MN array displayed good structural uniformity as marked by the arrow (* to *'). (c) SEM images for the cross-sectional view showing a double-layered structure of the MN consisting of an outer PS-PAA and an inner PS layer.

2. Material and methods

2.1. Fabrication of bullet-shaped double-layered MN patches

Polystyrene-*block*-poly(*tert*-butyl acrylate) (M_n: 28,000–123,000 g mol^{-1}; Polymer Source Inc., Canada) was hydrolysed in dichloromethane with trifluoroacetic acid as a catalyst for 24 h to obtain polystyrene-*block*-poly(acrylic acid) (PS-PAA) for use in the swellable MN tips. PS-PAA was then precipitated in excessive hexane, filtered, and washed several times to remove any trace of the catalyst, as described previously [28]. The female PDMS moulds (Sylgard 184, Dow Corning) for the MN array (10 × 10 MNs in 1 cm^2) were obtained from a bullet-shaped metal MN array, which was milled using a computer numerically controlled (CNC) machine. The first layer of the MN patch was prepared by solvent-casting of a 10 wt% PS-PAA solution in dimethylformamide (DMF) on the female PDMS mould with a 10 × 10 array of bullet-shaped cavities. A volume of 0.3 mL of the PS-PAA solution was slowly pipetted on the PDMS mould (1 cm^2) and then dried at 25 °C for 48 h after degassing under vacuum. Drying of the solvent prompted the formation of a thick film (~450 μm) at the tip region of the bullet-shaped cavity and a thin film (~150 μm) on the remainder of the mould. The remaining DMF in the dried PS-PAA layer was removed under vacuum at 140 °C for 30 min. To form the MN inner