Acta Biomaterialia xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

Dissolving polymer microneedle patches for rapid and efficient transdermal delivery of insulin to diabetic rats

Ming-Hung Ling, Mei-Chin Chen *

Department of Chemical Engineering, National Cheng Kung University, Tainan 70101, Taiwan

ARTICLE INFO

Article history:
Received 28 March 2013
Received in revised form 4 June 2013
Accepted 19 June 2013
Available online xxxx

Keywords:
Bioavailability
Bolus release
Hypoglycemic effect
Polysaccharide
Protein delivery

ABSTRACT

This study presents a dissolving microneedle patch, composed of starch and gelatin, for the rapid and efficient transdermal delivery of insulin. The microneedles completely dissolve after insertion into the skin for 5 min, quickly releasing their encapsulated payload into the skin. A histological examination shows that the microneedles have sufficient mechanical strength to be inserted in vitro into porcine skin to a depth of approximately 200 μm and in vivo into rat skin to 200–250 μm depth. This penetration depth does not induce notable skin irritation or pain sensation. To evaluate the feasibility of using these dissolving microneedles for diabetes treatment insulin-loaded microneedles were administered to diabetic rats using a homemade applicator. Pharmacodynamic and pharmacokinetic results show a similar hypoglycemic effect in rats receiving insulin-loaded microneedles and a subcutaneous injection of insulin. The relative pharmacological availability and relative bioavailability of insulin were both approximately 92%, demonstrating that insulin retains its pharmacological activity after encapsulation and release from the microneedles. Storage stability analysis confirms that more than 90% of the insulin remained in the microneedles even after storage at 25 or 37 °C for 1 month. These results confirm that the proposed starch/gelatin microneedles enable stable encapsulation of bioactive molecules and have great potential for transdermal delivery of protein drugs in a relatively painless, rapid, and convenient manner.

1. Introduction

Insulin is the most effective medicine currently available to control blood glucose levels in diabetic patients. Because of poor oral bioavailability and the current lack of alternative delivery routes subcutaneous (SC) injection of insulin remains the preferred approach to achieving the desired therapeutic effect. However, SC injection is inconvenient and painful, often leading to poor patient compliance [1]. The intradermal delivery of insulin is an attractive alternative because it is less invasive and less painful than SC delivery [2]. Intradermal delivery improves patient compliance and avoids drug degradation within the gastrointestinal tract and active drug loss due to first pass hepatic metabolism [3].

However, the outermost stratum corneum of the skin is an effective barrier against the entry of foreign proteins [4]. Microneedle technology provides an attractive method to create reversible microchannels in the skin [5], thereby increasing its permeability and enabling the delivery of a broad range of biotherapeutics that cannot permeate intact skin. The application of microneedles to the skin causes significantly less pain and tissue damage than a 26G hypodermic needle because of their micron sized dimensions [6].

The transdermal delivery of protein drugs using microneedles, which are made from dissolving or biodegradable polymers, has recently received great attention [7–10]. The advantage of polymer microneedles is that they can be produced less expensively than silicon microneedles and they do not pose a safety concern if they break off in the skin. This is because the needles can completely and safely dissolve or degrade within the skin. These microneedles are also unusable after removal from a patient's skin, thus significantly reducing infection transmission [11]. Unlike coated microneedles made of metal or silicon, drugs can be encapsulated within the polymer matrix of microneedles, increasing their drug loading capacity in one convenient formulation.

This study presents a dissolving microneedle patch, composed of starch and gelatin, that can rapidly dissolve in the interstitial fluid of the skin after insertion. These microneedles release their encapsulated insulin as they dissolve (Fig. 1). Starch is a naturally occurring, non-cytotoxic, and biodegradable polysaccharide that has been used as SC implants and drug carriers [12–16]. Starch has a long tradition as an excipient for solid dosage formulations, and can be used as filler, disintegrant, and binder [17]. Its low cost, biodegradability, non-polluting nature, and renewability make it a suitable candidate for developing sustainable materials in the pharmaceutical industry [13,18]. However, pure starch is a rigid and brittle polymer with poor film-forming properties [19]. Thus, physical blending with other biomaterials is a convenient and

* Corresponding author. Tel.: +886 6 2757575/62696; fax: +886 6 234 4496.
E-mail address: kokola@mail.ncku.edu.tw (M.-C. Chen).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.06.029

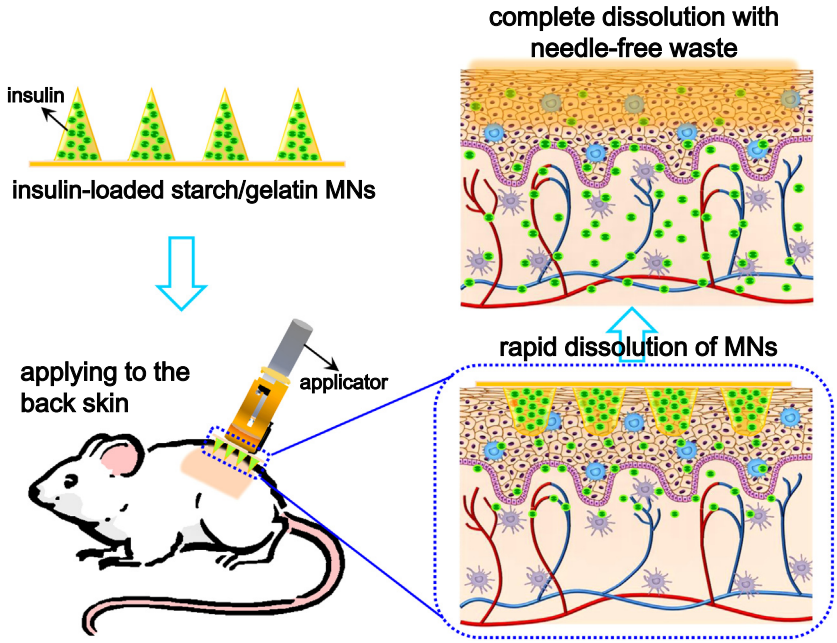

Fig. 1. Schematic illustration of transdermal delivery of insulin using starch/gelatin microneedles (MNs), which can rapidly dissolve in the skin to release encapsulated insulin.

effective method of improving the mechanical functionality of starch.

In this study starch was blended with gelatin, a hydrophilic protein derived from porcine skin, to improve its processability and produce a tough and strong composite suitable for needle material. Gelatin is widely used in pharmaceutical and medical applications because of its biodegradability, biocompatibility, non-immunogenicity, and film-forming ability [20]. These beneficial properties of gelatin have contributed to its proven record of safety as an ingredient in drug formulations [21,22] and a stabilizer in vaccines and other biopharmaceuticals [23].

To prevent the deleterious effects of high temperature or organic solvents on fragile protein molecules we used a mild solvent casting process for microneedle fabrication. This study also investigates the skin insertion abilities and in vitro transdermal drug delivery properties of the fabricated microneedles. We also measured the biological activity and long-term storage stability of insulin encapsulated in the microneedles. To assess the efficacy of using starch/gelatin microneedles for transdermal delivery of insulin we applied insulin-loaded microneedles to streptozotocin-induced diabetic rats using an in-house made applicator and investigated the pharmacokinetics and pharmacodynamics of microneedle-based insulin delivery and SC injection of insulin.

2. Materials and methods

2.1. Materials

Gelatin (from porcine skin, type A, 90–110 Bloom), fluorescein 5(6)-isothiocyanate (FITC) (molecular weight (MW) 389 Da), fluorescein isothiocyanate-dextran (FITC-dextran) (MW 2000 kDa), streptozotocin, citric acid, and insulin (from bovine pancreas, $\geqslant$25 U mg^{-1}, MW approximately 5.8 kDa) were purchased from Sigma–Aldrich (St Louis, MO). Starch (from wheat), an insulin ELISA kit, and a Glucose CII-Test kit were purchased from Merck Chemical Co. (Darmstadt, Germany), Mercodia AB (Uppsala, Sweden), and Wako Pure Chemical Industries (Osaka, Japan), respectively. Polydimethylsiloxane (PDMS) (Sylgard 184) and the Optimum Cutting Temperature (OCT) compound were purchased from Dow Corning (Midland, MI) and Tissue-Tek (Sakura Finetek, Torrance, CA), respectively. All chemicals were used as received without additional treatment.

2.2. Synthesis of FITC–insulin

To visualize drug diffusion in the skin insulin was fluorescently labeled with FITC using the methods described in the literature [24]. To remove the unconjugated FITC the synthesized FITC–insulin was dialyzed in the dark against deionized (DI) water, which was replaced daily until no fluorescence was detected in the supernatant. The solution was then lyophilized to obtain FITC-labeled insulin dry powder for the encapsulation process.

2.3. Fabrication of drug-loaded microneedles

A pyramidal microneedle master structure was created using an electro-discharge machining process (Micropoint Technologies Pte Ltd, Singapore). Microneedle molds were made from polydimethylsiloxane (PDMS) to inverse replicate the master structure, following a published procedure [10]. The PDMS molds obtained were repeatedly used to make polymer microneedles.

A two-step casting process was used to mold drug-loaded microneedle patches. A mixture of starch and gelatin at a weight ratio of 1:1 was dissolved in DI water with heating in a water bath at 90 °C for 30 min, and then cooled to room temperature to obtain a 10% (w/v) starch/gelatin gel. 2 mg of insulin or FITC–insulin dissolved in 0.3 ml of 0.1 M HCl solution was added to the 10% (w/v) starch/gelatin gel (10 ml) and mixed well to form a starch/gelatin gel containing insulin (pH $\approx$6.0). Approximately 200 mg of the drug-loaded gel was applied to the PDMS mold as the first layer and centrifuged in a swinging bucket rotor (221.12 V03, Hermle Labortechnik GmbH, Wehingen, Germany) at 5100 r.p.m. (3880g) at 20 °C for up to 1 h to fill the microneedle mold cavities. This horizontal centrifugation helped push the viscous gel into the mold holes. Gel remaining on the mold surface was removed and saved for future use. A second layer of starch/gelatin gel without drugs

Please cite this article in press as: Ling M-H, Chen M-C. Dissolving polymer microneedle patches for rapid and efficient transdermal delivery of insulin to diabetic rats. Acta Biomater (2013), http://dx.doi.org/10.1016/j.actbio.2013.06.029