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Microneedles fabricated from alginate and maltose for transdermal delivery of insulin on diabetic rats

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ABSTRACT

To reduce the inconvenient and painful of subcutaneous needle injection, the calcium ion cross-linked alginate/maltose (Ca^{2+}/Alg-Mal) composite microneedles have been fabricated by a template method. The as-prepared microneedles exhibited strong mechanical properties with the highest failure force around 0.41 N/needle. The biological activity and stability of loaded insulin in microneedles were investigated. Due to the good mechanical properties and excellent biocompatibility, the as-prepared microneedles have been applied for transdermal delivery of insulin on diabetic Sprague-Dawley (SD) rat models *in vivo*. After transdermal administration to the diabetic rats, the released insulin from biodegradable composite microneedles exhibit an obvious and effective hypoglycemic effect with relative pharmacological availability (RPA) and relative bioavailability (RBA) at 94.1 ± 5.6% and 93.7 ± 4.7% compared with that of subcutaneous injection route. This work suggests that as-prepared Ca^{2+}/Alg-Mal microneedles can be used to encapsulate insulin and have a potential application in diabetes treatment via transdermal ingestion.

1. Introduction

Diabetes mellitus is one type of metabolic diseases characterized by imbalance of blood glucose level regulation mechanisms, [1,2] and is becoming a worldwide public health problem. According to a report from International Diabetes Federation (IDF), as of 2015, 415 million adults were suffered from diabetes and the number is calculated to be 642 million by 2040 [3]. Insulin is the most effective medicine available to control blood glucose levels for tape 1 diabetic patients at present. The subcutaneous injection (SC) of insulin is a preferred drug-delivery route because of the poor oral bioavailability [4]. However, it is inconvenient and painful, often leading to poor patient compliance [5]. Many researches have been done to explore the more convenient and palatable routes for insulin administration, such as intranasal, [6] intrapulmonary [7], and oral [8–11]. For example, stimuli-sensitive microgels have been utilized for the development of self-regulated insulin delivery systems. These systems release insulin when they are induced to swell under the application of either a glucose or temperature stimuli [12–16]. Insulin can also be loaded into mesoporous silica particles for controlled delivery [17,18]. Nevertheless, the low and erratic bioavailability is limiting the development of polymer-based particulate system [19]. It remains a challenge to develop new delivery systems that are able to release insulin in a convenient manner, in order to allay patient discomfort and the inconvenience of multiple injections every day [20–22].

Microneedle provides an attractive method to create reversible microchannels in the skin for transdermal delivery of foreign proteins that cannot permeate intact skin [23]. The drug administration by microneedles shows less pain and tissue damage than 26G hypodermic needle due to the micron sized dimensions [24]. The microneedle delivery systems made by dissolving or biodegradable polymers are receiving gradually attention because of the completely dissolution and safely degradation within the skin [25–29]. The sodium alginate is a natural polymer with biodegradability, biocompatibility, and film-forming ability as a result of widely usage in pharmaceutical and medical applications [30–32]. Nevertheless, pure sodium alginate microneedles exhibit a poor mechanical property. Alginate cross-linked by calcium ion is used to strengthen the mechanical properties. However, the strength of cross-linked alginate microneedles is still not enough to pierce the skin. The aqueous solution of alginate is able to be mixed with a number of water-soluble materials, which may provide a possibility that the strength of alginate microneedles would increase by adding an appropriate amount of polysaccharide [33]. Sucrose also has been used to complex with gelatin or carboxymethyl cellulose for

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fabrication of the microneedles with enough strength due to a large number of hydroxyl groups in sucrose [34,35].

Maltose is a naturally carbohydrate of non-cytotoxic and biodegradable made from starch which used as filler and binder in SC implants and drug carriers [36–40]. It has the similar chemical structure with sucrose. In this study, maltose was added into cross-linked alginate microneedles to enhance the mechanical property. The mechanical strength for penetrate skin and *in vitro* drug release property of microneedles were tested. The biological activity and stability of loaded insulin in microneedles were investigated. The *in vivo* transdermal delivery of insulin using diabetic rats as models treated by insulin-loaded microneedles was evaluated.

2. Materials and methods

2.1. Materials

Sodium alginate (Alg, MW 32–250 kDa), insulin (from porcine pancreas, $\geq 27\ U{\cdot}mg^{-1}$, MW approximately 5.78 kDa), Fluorescein isothiocyanate isomer I (FITC, MW 389.38 Da) and streptozocin (STZ) were purchased from Aladdin (Shanghai, China). Calcium chloride anhydrous ($CaCl_2$) and D-(+)-Maltose monohydrate ($C_{12}H_{22}O_{11}{\cdot}H_2O$, MW 360.31 Da) were purchased from Macklin (Shanghai, China). Polydimethylsiloxane (PDMS, Sylgard 184) was bought from Dow Corning (Midland, MI). 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), sodium citrate tribasic dihydrate and citric acid were bought from Sigma-Aldrich (St. Louis, MO, USA). HeLa cells were offered by Shanghai cell bank of Chinese Academy of Sciences. To visualize the diffusion of insulin in the skin, the insulin had been labeled by FITC with the method described in the literature [41–44]. Male Sprague-Dawley (SD) rats were supplied by the Zhejiang Academy of Medical Sciences (Hangzhou, China). All chemicals were used as received without additional treatment.

2.2. Fabrication of microneedles

A pyramidal microneedle patch was made by polymethyl methacrylate (PMMA) (Micropoint Technologies Pte Ltd., Singapore. Patch size: 8 mm × 8 mm, Array Size: 10 × 10, Needle Height: 800 μm, Needle Base: 300 μm, Needle Pitch: 500 μm) and used as the male mold. We reversed mold with polydimethylsiloxane (PDMS) to form the microneedle molds [45]. And the acquired female mold was reused to make polymer microneedles.

To prepare the calcium ion cross-linked alginate/maltose (Ca^{2+}/Alg-Mal) composites, sodium alginate powder was firstly dissolved in DI water at a weight ratio of 1:4 with the stir in a water bath at 60 °C until to gain the homogeneous solution. And then, the 15% (w/w) $CaCl_2$ solution was added slowly with rapid mixing to cross-link alginate ($CaCl_2$-to-Alg weight ratio = 1:10). To enhance the mechanical properties of composite microneedles, 15% (*w*/w) maltose monohydrate was added simultaneously into the sodium alginate solution to form precursor for preparation of paste. A two-step casing process was used to fabricate Ca^{2+}/Alg-Mal composite microneedles. Firstly, about 100 mg insulin-loaded microneedle matrix was poured onto the PDMS female mold and centrifuged by a refrigerated centrifuge at 10000 rpm in 20 °C for 10 min to fill up the holes of microneedle mold to form the microneedle arrays. Then, approximately 200 mg paste precursor (without insulin) was tiled on the first layer to fill the cavity completely to form the microneedle basement. For fabrication insulin-loaded microneedles, the preparation process was same as the method mentioned above besides adding 2 mg insulin or FITC-insulin into 4 g as microneedle matrix. The whole microneedle mold contained two layer was centrifuged at the same operating conditions as well. Finally, the dried insulin-loaded microneedle patch was demolded from PDMS female mold after dried in the exsiccator at room temperature overnight. To determine the dose of insulin loaded on the microneedles, a piece of microneedle patch was randomly chosen from the same series of microneedles and dissolved in 10 mL DI water completely, and then tested the solution's absorbance by ultraviolet-visible spectrophotometer (UV–vis) (UV1901PC, Shanghai Aucy Technology Instrument CO. LTD, China) at a wavelength of 276.5 nm, calculated the dose through the absorbance-concentration standard curve which was made well previously [46].

2.3. Mechanical strength test

The universal material testing machine (WDW-02, Tianchen Testing Machine Co. LTD., Jinan, China) was used to study the transdermal strength of Ca^{2+}/Alg-Mal microneedle patch. The Ca^{2+}/Alg-Mal microneedle patch was placed on the bottom circular plate workbench. The initial distance between the tip of microneedle patch and the top circular plate workbench was approximate 3 mm. Then, the top workbench moved uniform down to microneedle patch at 1 $mm{\cdot}min^{-1}$. The load and the displacement were recorded by testing machine every 0.01 s to obtain the stress-displacement curve. The mechanical property of three kinds of microneedle patches made by different material (pure sodium alginate: Alg; sodium alginate and $CaCl_2$: Ca^{2+}/Alg; sodium alginate, $CaCl_2$ and maltose: Ca^{2+}/Alg-Mal) had been measured.

2.4. In vitro swelling test

To analyze the swelling of microneedle patch, the Ca^{2+}/Alg-Mal microneedle patch was placed in saturated steam environment at 37 °C to be observed the morphological changes. The instant morphological images at different moment (0, 5, 10, 20, 30, 40, 50, 60, 80 min) had been recorded.

2.5. Recovery of skin after microneedle treatment

To study the skin compatibility of Ca^{2+}/Alg-Mal microneedles, the recovery state of skin spot on a SD rat which been selected randomly was examined. Briefly, a SD rat was firstly shaved on its notum, and then penetrated by microneedle patch through pressing rapidly with thumb on the glabrous skin. Before that, the skin was cleaned by 75% alcohol and dried in air. Micrographs of the puncture marks on the skin were taken synchronously as a function of the time since the patch was removed from the skin, until the microholes were vanished completely.

2.6. In vitro cytotoxicity-test

To study the biocompatibility of microneedles, the colorimetric methyl thiazolyl tetrazolium (MTT) had been used *in vitro* cytotoxicity-test to analysis the cell proliferation. HeLa cells were cultivated in 96-well plate at a density of 6000 cells per well with 180 μL Dulbecco's modified eagle medium (DMEM), and the peripheral wells were filled by PBS buffer. The wells were grouped a column by a column according the different concentrations (0, 100, 200, 400, 800 and 1000 μg/mL) of Ca^{2+}/Alg-Mal composite solution and each group embraced five wells. The filled 96-well plates were cultured in a CO_2 (5%) incubator at 37 °C for 48 h, 20 μL 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium solution (MTT solution, 5 mg/mL) was added in each well. And the mixed wells were cultured for an additional 4 h. After that, the DMEM were removed from wells, and then 150 μL DMSO was added to each well. After oscilation for 10 min, the absorbance of well' solutions were recorded by microplate reader (Multiskan MK3, Thermo Electron Corporation) at a reference wavelength of 490 nm.

2.7. Stability of insulin encapsulated in microneedles

The biological activity of encapsulated insulin in Ca^{2+}/Alg-Mal microneedles was measured by an insulin ELISA kit. The insulin-loaded microneedles were firstly dissolved in DI water. Three groups of