Materials Science and Engineering C 71 (2017) 725–734

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Fabrication of biodegradable composite microneedles based on calcium sulfate and gelatin for transdermal delivery of insulin

Weijiang Yu [a], Guohua Jiang [a,b,c,*], Depeng Liu [a], Lei Li [a], Hua Chen [a], Yongkun Liu [a], Qin Huang [a], Zaizai Tong [a,b,c], Juming Yao [a,b,c], Xiangdong Kong [d]

[a] Department of Materials Engineering, Zhejiang Sci Tech University, Hangzhou 310018, China
[b] National Engineering Laboratory for Textile Fiber Materials and Processing Technology (Zhejiang), Hangzhou 310018, China
[c] Key Laboratory of Advanced Textile Materials and Manufacturing Technology (ATMT), Ministry of Education, Hangzhou 310018, China
[d] College of Life Science, Zhejiang Sci Tech University, Hangzhou 310018, China

ARTICLE INFO

Article history:
Received 1 August 2016
Received in revised form 12 October 2016
Accepted 24 October 2016
Available online 26 October 2016

Keywords:
Biodegradable
Microneedles
Insulin
Transdermal delivery
Hypoglycemic effect

ABSTRACT

To reduce the inconvenience and pain of subcutaneous needle injection, the calcium sulfate and gelatin biodegradable composite microneedle patches with high aspect-ratio microneedles (MNs) and a flexible substrate have been developed. The microneedles with an aspect-ratio approximate 6:1 exhibit excellent mechanical property which can achieve 0.4 N for each needle. The cross-section views show the inside of microneedles that have abundant pores and channels which offer potential for different drug-release profiles. The preparation procedures, degradable property for the biodegradable composite microneedle patches are described in the paper. Insulin, the drug to control blood glucose levels in diabetic patients, has been embedded into the biodegradable composite MNs. The hypoglycemic effect for transdermal delivery of insulin is studied using diabetic Sprague-Dawley (SD) rats as models *in vivo*. After transdermal administration to the diabetic rats, the released insulin from biodegradable composite MNs exhibit an obvious and effective hypoglycemic effect for longer time compared with that of subcutaneous injection route. This work suggests that biodegradable composite MNs containing of insulin have a potential application in diabetes treatment via transdermal ingestion.

1. Introduction

Diabetes is one of the leading lethal diseases in the world. According to a report from International Diabetes Federation (IDF), there are 415 million adults were living with diabetes in 2015 and this number is expected to increase to around 642 million or one in ten adults by 2040 [1]. Especially in the developing countries, it has become one of the most lethal diseases. Insulin is a peptide drug and the most effective medicine to control blood glucose levels in type I diabetic patients. Due to the poor absorption or enzymatic degradation of insulin in the gastrointestinal tract and liver, the subcutaneous needle injection is the preferred insulin administration method. However, it is inconvenient and painful, often leading to poor patient compliance [2]. During the past few years, many efforts have been devoted to explore more convenient and palatable routes for insulin administration, including intranasal [3], intrapulmonary [4], oral [5–7] and transdermal administration [2]. However, insulin has low oral bioavailability due to the degradation of insulin by proteolytic enzymes and acidic pH and the low penetration of insulin across the intestinal lining into the blood stream [8]. Polymer-based particulate systems have been widely investigated as a carrier to increase insulin absorption by protecting insulin from enzymatic degradation, increasing mucoadhesive ability, and enhancing transport across the mucosa [9–16]. However, low and erratic bioavailability remains major problems [17].

A recent study reported that compared with traditional subcutaneous (SC) drug administration, transdermal drug delivery using a microneedle resulted in less insertion pain and led to a faster onset and offset of insulin pharmacokinetics in body [18]. Usually, MNs can be defined as solid or hollow cannula with an approximate length of 50–900 μm and an external diameter of not >300 μm [19]. Biodegradable MNs are widely studied because they can achieve extended drug release. MNs composed of biodegradable materials have higher drug payloads and no potential biohazardous waste after use [20]. Most biodegradable cMNs are made of water-soluble polymers, which dissolve and release the drug molecules after contact with interstitial fluid in the skin. For example, Jung et al. [21] reported to prepare dissolving MNs for delivery of lipophilic drugs using hyaluronic acid (HA) and polyvinyl pyrrolidone (PVP) based on drawing lithography. The phase transition of powder form of lipophilic drugs due to interior chemical bonds between drugs and biodegradable polymers and formation of nano-sized colloidal structures allowed the fabrication of dissolving

* Corresponding author.
E-mail address: ghjiang_cn@zstu.edu.cn (G. Jiang).

http://dx.doi.org/10.1016/j.msec.2016.10.063
0928-4931/

microneedles (DMNs) to generate a powerful transdermal drug delivery system. Recently, fabrication of polymeric MNs based on direct photolithography, without any etching or molding process, were reported by Dardano et al. [22] Polyethylene glycol, casted into a silicone vessel and exposed to ultraviolet light through a mask, cross-links when added by a commercial photocatalyzer. By changing the position of the MNs support with respect to the vessel, different shapes and lengths can be achieved. The merits of low-cost, easy-fabrication, and FDA-approved biodegradable poly-ε-caprolactone (PCL) and dissolving poly(ethyleneglycol) (PEG) also have been developed for preparing microneedle patches [23]. However, most of these microneedle polymers have challenges in terms of mechanical strength and stability. The high-temperature processing or use of organic solvents for loading drugs limits the use of the synthetic biodegradable polymers. Metallic MNs also suffer from the limitation of drug loading amount based on a coating method [24].

On other hand, a flexible substrate in the microneedle array is preferred to assist drug delivery and skin penetration. Due to the elasticity and toughness of the skin, microneedle arrays with a rigid substrate often encounter the "bed of nail" effect, that is, the force distributed on each needle, which reduces the efficiency of insertion. Therefore, some MNs are not inserted, and others are pulled out with the movement of the substrate. Both can lead to insufficient and inconsistent drug delivery and waste. MNs with a flexible substrate can fit furrowed and deformable skin and thus improve the efficiency of breaching the stratum corneum [20].

Therefore, development of a suitable material for a microneedle array is significantly driven by the rapidly increasing interest in the field of transdermal drug delivery with minimal invasion. Ceramics could be promising in this respect, because of their advantageous properties; they are mechanically strong and biocompatible, and they offer controlled porosity and easy handling during the production process. Their adjustable porosity and the electrostatic interaction between the ceramic surface and the transported drugs have been widely studied and utilized in a broad range of biomedical applications to control drug release [25]. Our aim in this study is to improve biodegradable composite microneedle patches with higher-aspect-ratio needles and a flexible and self-swelling substrate. The substrate is designed not only to act as a supporting layer but also to assist drug delivery. After contact with body fluid, the substrate would separate from the needle, leaving the needles inside the skin as drug depots. This design is intended to improve the skin penetration and modify the drug release behavior of the biodegradable composite microneedle array.

2. Experimental

2.1. Materials and animals

Calcium sulfate hemihydrate ($CaSO_4 \cdot 0.5H_2O$, particle size < 100 nm) was purchased from Acros (Geel, Belgium). Gelatin (from porcine skin, type A, 100 Bloom), rhodamine B, glutaraldehyde solution, insulin (from porcine pancreas, ≥27 units/mg, M_W approximately 5.78 kDa) and fluorescein isothiocyanate isomer I (FITC) (M_W = 389 Da) were purchased from Aladdin (Shanghai, China). Polydimethylsiloxane (PDMS; Sylgard 184) was purchased 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 purchased from Sigma-Aldrich (St. Louis, MO, USA). RAW 246.7 cells were offered by Shanghai cell bank of Chinese Academy of Sciences. Male Sprague-Dawley (SD) rats and bar like feed were supplied by the Zhejiang Academy of Medical Sciences (Hangzhou, China).

2.2. Synthesis of FITC labeled insulin

To observe the diffusion of insulin, FITC was conjugated to insulin using the method described previously [26–28]. In brief, a solution of FITC in dimethylsulfoxide (4.5 mg/mL) was slowly dripped into a 5 mL insulin solution (4 mg/mL, 0.1 M Na_2CO_3). After reacting for 12 h at 4 °C, 10 mL NH_4Cl (50 mM) was added to terminate the coupling reaction. The mixture was stirred for 2 h at 4 °C. Unconjugated FITC was separated by dialysis against deionized (DI) water, which was changed every 4 h, using a 3500 kDa dialysis tube. The FITC labeled insulin was freeze-dried at −80 °C and stored at 4 °C in the dark until further use.

2.3. Fabrication of insulin-embedded MNs

A commercial microneedle stamp was used as a master template. The microneedle stamp consisted of 80 (8 × 10 array) conical titanium alloy needles with a length of approximately 900 μm, and a base radius of 150 μm. To inverse-replicate the master template, PDMS solution with curing agent added was poured over the master template, after carrying out for 30 min under vacuum of ~0.1 MPa to remove air bubbles, next curing at 85 °C for 1.5 h. Subsequently, the cured negative molds were harvested by peeling from the master template discreetly.

To synthesize the gelatin/calcium sulfate hemihydrate (GelCS) composites, gelatin powder was firstly dissolved in deionized (DI) water to obtain 50% (w/v) homogeneous solution in a water bath at 50 °C for 1 h. Following calcium sulfate hemihydrate powder was added to gelatin solution to form a paste (powder-to-liquid weight ratio = 1:5), and calcium sulfate hemihydrate as inorganic part in the composite is to obtain the mechanical strength. Next, the paste (3 g) kept stirring at 37 °C in a beaker with 2 mg insulin or FITC labeled insulin and 125 μL 1.25% aqueous glutaraldehyde solutions (0.05 wt% in order to avoid excessive biological toxicity) [29] for further blending. Glutaraldehyde was used for further increasing the mechanical strength of GelCS composites by crosslinking the gelatin.

Before the casting process, GelCS composites, centrifuge tubes and PDMS molds should be preheated at 37 °C to ensure the GelCS composites in a liquid state over the molds. Approximately 300 mg of GelCS composites were tiled on the PDMS molds and centrifuged at 8000 rpm. at 30 °C for 20 min. Residual material can be collected for recycling. Then PDMS molds with GelCS composites loaded were dried in desiccators for at least 3 h. GelCS microneedle arrays with a thin film at the base were created from this process. A transparent tape (Biaxially Oriented Polypropylene; BOPP) was then applied to the back of the film. The transparent tape was carefully peeled away from the PDMS mold. Therefore the entire microneedle patch was removed from the PDMS mold. All microneedle patches were sealed up in dark storage in the desiccators at room temperature. To investigate the dose of insulin embedded in the microneedle patches, a microneedle patch was randomly selected the patches and immersed in 10 mL H_2O to dissolve the MNs completely. The dose of embedded insulin was determined by insulin ELISA kits.

2.4. Mechanical strength test

The universal material testing machine (WDW-02, TIANCHEN Testing Machine Co. LTD., Jinan, China) was utilized to study the mechanical strength of GelCS microneedle patches. The GelCS microneedle patch was fixed on the aluminum base plate. The initial distance between the base of GelCS microneedle patch and top aluminum plate was set as 3 mm. The velocity of the top aluminum plate moving toward the GelCS microneedle patch was set at 0.2 $mm \cdot s^{-1}$. In the test, instantaneous force and displacement were recorded by testing machine every 0.05 s to get the force displacement curve. In addition, weights of 10 g, 20 g, 50 g, 100 g, 150 g, 250 g, and 500 g were exerted on the tips of microneedle patch to explore the deformation.

2.5. MTT analysis

The *in vitro* cell proliferation analysis was measured using a colorimetric methyl thiazolyl tetrazolium (MTT) assay in RAW cells. In brief,