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Polymer microneedles fabricated from alginate and hyaluronate for transdermal delivery of insulin

Weijiang Yu [a], Guohua Jiang [a,b,c,*], Yang Zhang [a], Depeng Liu [a], Bin Xu [a], Junyi Zhou [a]

[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

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ABSTRACT

To reduce the inconvenient and painful of subcutaneous needle injection, the polymer microneedle patches that fabricated from modified alginate and hyaluronate were prepared for transdermal delivery of insulin. The as-prepared microneedles (MNs) exhibited excellent mechanical strength to penetrate the skin and good degradability to release loaded insulin. *In vitro* skin insertion capability was determined by staining with tissue-marking dye after insertion, and the real-time penetration depth was monitored using optical coherence tomography. Confocal microscopy images revealed that the rhodamine B and fluorescein isothiocyanate-labeled insulin (FITC-insulin) can gradually diffuse from the puncture sites to deeper tissue. *In vivo* and pharmacodynamic studies were then conducted to estimate the feasibility of the administration of insulin-loaded microneedle patches on diabetic mice for glucose regulation. The relative pharmacologic availability (RPA) and relative bioavailability (RBA) of insulin from microneedle patches were 90.5 ± 6.8% and 92.9 ± 7%, respectively. These results suggests the MNs developed in this study have a promising application in diabetes treatment *via* transdermal delivery.

1. Introduction

Diabetes mellitus, as one of the oldest diseases, has been a concern for a long time in the world. Effective treatments have been improving since the early part of the 20th century [1–4]. Although great progress has been achieved on diabetes in recent decades, daily insulin injections may disrupt the diabetic patient's daily life. The traditional insulin injections usually use syringes fitted with a hypodermic needle, which provides a simple, rapid and low-cost way to deliver drugs but easily generate sharp biohazardous waste [5]. However, the pain can be a particular challenge for patients' compliance, especially for children. Therefore, many new approaches have been developed to replace the hypodermic injection (including subcutaneous injection, intravenous injection, *etc.*), *e.g.*, needlefree high pressure injection system [6], microparticles for inhalation [7,8], nanoparticles for oral administration [9–13]. Among them, the transdermal drug delivery has been received the intense investigation due to less pain and tissue damage [14–22]. MNs for drug delivery are the main research directions of transdermal drug delivery now, including solid MNs [14], coated MNs [15,16], hollow MNs [17,18] and dissolvable (biodegradable) MNs [19,20].

MNs, micron sized needles, are of great interest to scientists as a new therapeutic vehicle through transdermal routes, especially for vaccines, drugs, small molecules [21]. As a device of transdermal drug delivery, it offers a painless and minimally invasive approach to pierce the outermost skin layer, the stratum corneum, with its microscopic needles [22]. MNs made from dissolving or biodegradable polymers have recently received great attention. They can be completely and safely dissolved or degraded within the skin. These MNs are also unusable after removal from a patient's skin, thus significantly reducing infection transmission. The drugs can be encapsulated within the polymer matrix of MNs, increasing their drug loading capacity in one convenient formulation [23].

This study presents a dissolving polymer microneedle patch, composed of 3-aminophenylboronic acid-modified alginate (Alg-APBA) and hyaluronate (HA) that can rapidly dissolve in the interstitial fluid of skin after insertion. These MNs release their encapsulated insulin as they dissolve. Both of alginate (Alg) and HA are naturally occurring, non-cytotoxic, and biodegradable polysaccharides, and suitable candidates for developing sustainable materials in the pharmaceutical industry [24]. In addition to the naturally occurring and nontoxic properties, alginate and hyaluronate possess cross-linked characteristics for improving the mechanical functionality of them.

In this study, Alg was chemically modified by 3-aminophenylboronic acid to prepare Alg-APBA which can form linkages with glucose and realize self-regulated release of insulin [25]. Then, chemical cross-linking

* Corresponding author at: Department of Materials Engineering, Zhejiang Sci Tech University, Hangzhou 310018, China.
E-mail address: ghjiang_cn@zstu.edu.cn (G. Jiang).

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of Alg-APBA and HA was carried out to prepare a new material for the encapsulation of insulin as a form of MNs in the presence of Ca^{2+} ions. The crosslinked structure was designed to provide excellent mechanical strength to penetrate the skin and extended release time for encapsulated drugs to diffuse into skin issue after absorption of the interstitial fluid. To demonstrate the utility of these MNs, drugs delivery experiments were performed. Successful delivery of dyes and insulin into mice skin *in vitro* and *in vivo* was demonstrated. The relative pharmacodynamic and pharmacokinetic parameters were analyzed as well. This study demonstrated that the proposed microneedle system featuring this unique design allows more convenient and efficient self-administration of drugs into the skin.

2. Experimental

2.1. Materials

3-Aminophenylboronic acid (APBA), 1-(3-dimethyl aminopropyl)-3-ethylcarbo diimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), sodium alginate (Alg), sodium hyaluronate (HA), calcium chloride ($CaCl_2$), rhodamine B, insulin (from porcine pancreas, ≥27 units/mg, MW approximately 5.78 kDa), fluorescein isothiocyanate isomer I (FITC) (MW = 389 Da), streptozotocin (STZ) were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). Optimum cutting temperature (OCT) compounds were purchased from Tissue-Tek (Sakura Finetek, Torrance, CA). Isoflurane was purchased from RWD Life Science Corporation (Shenzhen, China). Insulin ELISA kit was purchased from DuMa Biotechnology Co., Ltd. (Shanghai, China). Polydimethylsiloxane (PDMS@Sylgard 184) was purchased from Dow Corning (Midland, MI). Hela cells were gained from Institute of Biochemistry and Cell Biology (Shanghai, China). They were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum and 1% non-essential amino, sodium citrate tribasic dehydrate and citric acid were purchased from (Sigma-Aldrich, China). Spague Dawley (SD) male rats (200 ± 20 g) were obtained from Zhejiang Academy of Medical Sciences (Hangzhou, China). Poly(methyl methacrylate) (PMMA) MNs male molds were purchased from Micropoint Technologies Pte, Ltd. (Singapore). All chemicals were used as received without additional treatment.

2.2. Fabrication of Alg-APBA/HA microneedle patches

Alg-APBA was firstly synthesized according to the procedure previously reported [26,27]. The detial synthesis process had been included in Supporting Information. According to the integral date of NMR, the substitution degree of APBA was ~25%. MNs were fabricated using PDMS micromold and the molding processes were described previously [28]. Briefly, the master mold consists of 100 (10 × 10) pyramids-shaped MNs and each microneedle tapers from a square with a side length of ~300 μm to a tip measuring ~10 μm in width. The height of microneedle is ~700 μm and the space between each two MNs is ~600 μm. To obtain Alg-APBA/HA microneedle patches, Alg-APBA was firstly dissolved in DI water with a weight ratio at 1:5. To afford sufficient viscosity for centrifugation, sodium hyaluronate ($W_{Alg\text{-}APBA}$: W_{HA} = 1: 1) was then mixed with Alg-APBA solution directly to form composites paste. Finally, 1.0% $CaCl_2$ aqueous solution was added to chelate with the alginate and hyaluronate, which was used for crosslinking the polymer to increase the mechanical strength of MNs [29–31]. Rhodamine B, insulin or FITC-insulin was added in the mixing process. The formed paste with drug loaded (~100 mg) was firstly applied to the PDMS mold, centrifugation (8000 rpm for 3 min) pushed the composites into the bottom of microholes in the PDMS mold, after removing redundant material, no drug loaded composites (~300 mg) was filled in the female mold for another centrifugation (8000 rpm for 5 min). These fabricated microneedle patches were dried in ambient environment overnight. And Alg-APBA/HA microneedle patches were stored in desiccator keeping in dark place after being peeled off from female molds.

2.3. Mechanical strength test and degradation experiments

Compression testing was performed by a universal, static testing system (5943 single column materials testing system, Instron Co., Ltd. USA). In order to compare the mechanical strength of different MNs, we pressed Ca^{2+} crosslinked Alg-APBA/HA, no crosslinked Alg/HA and Alg-APBA/HA MNs patches against a stainless steel plate to obtain the force-displacement curves. The initial distance between the base of microneedle arrays and top stainless steel plate was set as 1.5 mm. The velocity of the top stainless steel plate moving toward the microneedle arrays was set at 0.5 $mm \cdot s^{-1}$. In the test, instantaneous force and displacement have been recorded by testing machine every 0.1 s since the needle contacted the stainless steel plate. In addition, weights of 10 g, 50 g, 100 g, 250 g and 500 g were exerted on the tips of microneedle arrays to explore the deformation. To demonstrate the degradation of Alg-APBA/HA microneedle patches, the patches are sticked on a polyester film and incubated in an airtight and saturated steam environment at 37 °C for 3 h. The degradation images of different incubation times are collected by a digital camera.

2.4. Ex vivo skin insertion

In order to explore the skin condition after insertion *in vivo*, only rhodamine B or FITC-insulin loaded Alg-APBA/HA microneedle patches were applied on the living rats for 5 min. Before insertion, the hair of SD rats' back was removed by an electric shaver. The bare skin was cleaned by 75% alcohol and dried in the air. After the rhodamine B loaded Alg-APBA/HA microneedle patches applied for 5 min, the skin was observed by a digital camera and a laser confocal scanning microscope to explore the insertion ratio and dyes diffusion. *In vivo* fluorescence images with FITC-insulin loaded Alg-APBA/HA microneedle patches were obtained using an *in vivo* imaging system (IVIS KINEXC, Caliper Corporation, USA). The SD rats were anaesthetized using Isoflurane. The recovery of the skin was recorded by a digital camera until the marks completely disappeared.

To evaluate the insertion ability of Alg-APBA/HA microneedle patches *in vitro*, rhodamine B loaded or FITC-insulin loaded Alg-APBA/HA microneedle patches were applied on the separated rat skins. FITC was conjugated to insulin using the same way previously [32–34]. Before the experiment, the rats' skins were cleaned with 75% alcohol and dried for 10 min after wetting in 37 °C water. To observe the diffusion of the drug loaded in the MNs over time, rhodamine B and FITC-loaded microneedle patches were removed from the skins after applying for 5 min. The skin samples were collected and sheared immediately. Areas with application sites were embedded in an OCT compounds for histological sectioning on a cryotome (CryoStar NX50, Thermo Fisher Scientific, USA). These frozen specimens were sliced into 7-μm-thick sections to be observed under a confocal laser scanning microscope (C2, Nikon Corporation, Japan). The insertion depth of MNs and diffusion of rhodamine B (red) and FITC-insulin (green) can be observed from the bright field and fluorescence images directly.

For better reappearing the subcutaneous diffusion, complete skin samples of FITC-insulin loaded microneedle patches applying for 5 min and rhodamine B loaded microneedle patches applying for 12 h were scanned in different z-axis height and 3D reconstructed by confocal laser scanning microscope (CLSM) at a excitation wavelength of 488 nm (FITC) and 543 nm (rhodamine B). After determining the maximum and minimum value of z axis height in CLSM, images were obtained from xy-plane with step increase of z-axis height. 3D reconstruction images were obtained by overlapping xy-plane images in different z-axis height.