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Application of composite dissolving microneedles with high drug loading ratio for rapid local anesthesia

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ABSTRACT

We report a type of dissolving microneedles (DMNs) which was made of composite matrix materials of hydroxypropyl methyl cellulose (HPMC) and poly(methylvinylether-*co*-maleic anhydride) (PMVE/MA copolymer, Gantrez S-97), and was successfully loaded with lidocaine hydrochloride. The weight of lidocaine hydrochloride loaded in the microneedles tip was 70% of the weight of the whole tip. The content was 3.43 ± 0.12 mg in weight, which was determined by high performance liquid chromatography (HPLC). The results for mechanical test showed that these microneedles were able to penetrate into the skin of the experimental animals, that was proved using organic staining, texture analyzer and histological examination. The fracture force of the microneedles was 5.442 ± 0.412 N, which was much higher than the one required for the skin penetration. The DMNs with lidocaine hydrochloride could be dissolved inside of the rat skin in 5 min. The onset time would be faster (in < 5 min) when it was applied to the guinea pig model, in comparing with a commercially available anesthesia cream that had an onset time for 100 min. However, the efficacy of the DMNs for the local anesthesia only lasted for 16 min. It was shorter than that of the commercially available anesthesia cream with which the efficacy could last for about 130 min. After the DMNs was packaged under the vacuum and dark condition, it was stable for 3 months under the condition of a temperature of 40 ± 2 °C and a humidity of 75 ± 5%. The result of the experiment for the safety evaluation showed that the microneedles were non-irritating and non-allergenic to the skin. In conclusion, the DMNs with lidocaine hydrochloride could be safely administered to the skin with a quick onset time for the local anesthesia.

1. Introduction

Together with the continuous improvement in the life-style, technology and medical sciences, the medical and health services are demanded for higher quality. The modern medicine calls for “painless medical procedures” that is not only the need for patients, but the future direction as well. Currently, many clinical procedures, including superficial operations on skin, such as skin biopsy, curettage, chemical peeling (Johann et al., 2018; Goodhead and Hampton, 2018; Erbil et al., 2007), and medical beauty, such as cosmetic-dermatology injection, laser treatment, and tattoo (Li et al., 2003; Yang et al., 2018; Lorgeou et al., 2017), may cause the discomfort, pain and anxiety. The patients' compliance may often be poor because of pain and stresses (anxiety, fear). The problems may be resolved by topical anesthesia in the area with the procedures. The ideal topical epidermal anesthesia may possess the characteristics such as fast onset, sufficient maintenance time, easy to use and less side effect (Votta-Velis et al., 2013). The current anesthetic method in the clinical uses the subcutaneous multipoint injection of local anesthetic drugs (Ata et al., 2016). However, the procedure *per se* may follow with pain and require professional anesthesia skills, while the external use of cream formulation (Horikiri et al., 2018; Sobbeler and Kastner, 2017) possesses the problems of slow onset, insufficient efficacy and others. Therefore, it is of important clinical significance to solve these problems in the development of topical epidermal anesthetic agents with painless, rapid onset, less side effect, and convenience.

Microneedles refer to an array of multiple micro-needles. Typically, a needle is a few tens of micrometers to a few millimeters in length and has a tip with several tens of micrometers or less in diameter (Amodwala et al., 2017). Microneedles is a kind of technology that functions with transdermal drug delivery with efficiency, safety and novelty that is beyond the reach of other traditional transdermal drug delivery technologies (González-Vázquez et al., 2017). First of all, microneedles break the major barrier in transdermal delivery - the stratum corneum, which not only greatly enhances the delivery efficiency of drugs, but also enables the delivery of the drugs that are

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macromolecular and hydrophilic. Secondly, due to its small structural features, microneedles can only penetrate the stratum corneum in the process of application without touching the nerves distributing on the dermis. This procedure achieves painless administration and improves patient compliance (Zhou et al., 2010). At present, many studies have investigated the preparation of microneedles with different materials (Ito et al., 2008; Serpe et al., 2016; Indermun et al., 2014), such as sugar, metal, silicon, polymers and hydrogels. There are also many different types of microneedles, such as hollow microneedles, solid microneedles, dissolving microneedles and swelling microneedles (Vander et al., 2018; Choi et al., 2013; Wang et al., 2015). However, these microneedles have several problems including poor biocompatibility and harmful residues inside of the skin from a fracture (Park et al., 2005). These problems have limited the clinical application of microneedles and hindered the progress of the marketing activity. We are reporting here a new kind of dissolving microneedles (DMNs) which uses dissolving or biodegradable materials, a type of self-degradable material, and expands the drug loading. In addition, the DMNs are made of different combinations of matrix materials that can achieve different properties for controlled drug releases (Kim et al., 2012).

Lidocaine is a commonly used local anesthetic drug in the clinic with an efficacy of the middle effect (Ribeiro et al., 2016). The literatures have reported many topical preparations with lidocaine formula including liposomes, flexible nanoliposomes, ethosomes, solid lipid nanoparticles, microemulsions, tinctures, microemulsion gels, liposome gel, gel and film (Kreilgaard et al., 2001; VanHal et al., 1996; Negi et al., 2016; Zhu et al., 2013; Repka et al., 2005). However, these external transdermal preparations have a long onset time, drug loading limitation, and cause significant drug waste. In order to overcome the problems in these topical preparations, we have developed the DMNs with a high drug loading of lidocaine hydrochloride for a rapid local anesthetics and a safe skin care.

2. Materials and methods

2.1. Materials

Lidocaine hydrochloride (99%) was purchased from Jizhou Pharmaceutical Co., Ltd. of Jiangsu. Acetonitrile (HPLC grade) was purchased from TEDIA USA. HPMC (10KD) was purchased from Tai'an Ruitai Cellulose Factory. Gantrez S-97 was purchased from Shanghai Juwei International Trade Co., Ltd. Polydimethylsiloxane (PDMS) was purchased from Dow Corning Corporation. Compound lidocaine cream was purchased from Beijing Ziguang Pharmaceutical Co., Ltd. Guinea pigs, rats and rabbits were provided by the Experimental Animal Center of Zhejiang Province. Exposed swine ear skin was obtained from the local slaughterhouse. All other materials were of analytical grade and used as received.

2.2. Methods

2.2.1. Preparation of DMNs

The microneedles female molds were prepared by mixing PDMS and curing agent at a mass ratio of 10:1. As shown in Fig. 1, the layered microneedles were prepared by a two-step casting method with 42 needles on each patch. The matrix materials for preparation of microneedles were selected in according to previous description (Zhang et al., 2017) with a little modification. The ductile material of HPMC and another brittle material of Gantrez S-97 were mixed with the drug and water at a mass ratio of 3% HPMC, 12% Gantrez S-97, 35% lidocaine hydrochloride and 50% deionized water to achieve the best mechanical properties. Then the solution was centrifugated (80-2, Shanghai Meixiang Instrument Limited Company, China) at 3500 rpm for 5 min to remove the bubbles. The 0.2 g solution was uniformly applied to the surface of the microneedles female mold and centrifuged (TDZ5-WS, Hunan Xiangyi Instrument Centrifuge Co., Ltd., China) at 3500 rpm for 5 min. Excess matrix solution at the surface was scrapped off to complete the preparation of the needle tip. The 0.2 g solution without lidocaine hydrochloride was prepared for the backing layer of the microneedles as the method described above. Microneedles were dried for 10 h at 40 °C. The morphology of the microneedles was inspected under a scanning electron microscope (SEM, S-4700 (II), Hitachi, Japan) after demolding.

2.2.2. Determination of lidocaine hydrochloride content in tips of the microneedles

Prepared lidocaine hydrochloride DMNs were dissolved in 5 mL of phosphate buffer solution and filtrated through a 0.45 μm microporous membrane filter. The 20 μL of filtrated solution was injected into the HPLC system equipped with automatic injection pump (515, Waters, USA), UV detector (486, Waters, USA), autosampler system (717, Waters, USA) and a reversed phase column (4.6 × 150 mm, Hypersil BDSC C18; Dalian Elite Analytical Instruments Co., Ltd., China). The mobile phase was a mixture of phosphate buffer solution (1.3 mL of 1 mol/L sodium dihydrogen phosphate solution was mixed with 32.5 mL of 0.5 mol/L sodium phosphate dibromide solution, and diluted to 1000 mL with water) with acetonitrile at a volume ratio of 55:45 and its pH was adjusted to 8.0. The column temperature was 25 °C and the flow rate was 1.0 mL/min. The lidocaine hydrochloride eluted from the column was detected at a wavelength of 263 nm. Running cycle in each assay was 7 min and the retention time of lidocaine hydrochloride was 4.8 min.

2.2.3. Mechanical characterization of the microneedles

The mechanical properties of lidocaine hydrochloride DMNs were investigated by the compression mode of texture analyzer (Larrañeta et al., 2014) (TA.XT Plus, SMS, UK). The moving speeds of P/6 probe for pre-test, test and post-test were setup at 1.5 mm/s, 0.5 mm/s and 1.5 mm/s, respectively. The trigger force was 0.06 N and data acquisition rate was 25. The DMNs was placed upward so that the needle was oriented in parallel to the axial direction of the probe. The pressure on the needle was recorded during probe displacement.

The same texture analyzer was combined with fresh pig ear skin to investigate the penetration force of microneedles. As shown in Fig. 3C, after fixing the pig ear skin with the Franz diffusion cell (filling foam block in the receiving room), the master mold of metal microneedles was placed on the pig ear skin with the needle vertically downward, aligning with the position of the probe. The parameters of the texture analyzer were setup as above. The pressure on the needle was recorded during probe displacement.

2.2.4. Experiment for skin puncture of the microneedles

A number of isolated skin from healthy and non-injured rats were used in the experiment. After removal of subcutaneous fat, the skin was stored in saline. In the experiment, the skin was spread on the foam (polyvinyl chloride, 2 cm in thickness) and the stratum corneum was out upward. Excess solution on skin surface was removed by using absorbent paper. The lidocaine hydrochloride DMNs was loaded on the applicator to carry out the vertical skin puncture. The microneedles were stayed on the skin for 3 min and the punctured skin was spared for use. The partially punctured skin was immediately stained with methylene blue solution and the excess methylene blue dye was removed with isopropanol. Then the stained skin was photographed. The remaining punctured skin was fixed in 10% formaldehyde solution and sliced with an automatic slicer (YD-355AT, Jinhua Yidi Medical Equipment Co., Ltd., China). The slice was stained with hematoxylin and eosin. The tissue sections were visualized and photographed on fluorescence inverted microscope (TI-S, Nikon Corp., Japan).

2.2.5. Experiment for intradermal dissolution of the microneedles

The back hairs of rat were removed after anesthesia. One piece of the rat back skin was divided into four areas. One microneedle was