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Novel sodium fusidate-loaded film-forming hydrogel with easy application and excellent wound healing

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ABSTRACT

To develop a novel sodium fusidate-loaded film-forming hydrogel (FFH) for easy application and excellent wound healing, various FFH formulations and corresponding FFH dried films were prepared with drug, polyvinylalcohol (PVA), polyvinylpyrrolidone (PVP), propylene glycol, ethanol and water, and their film forming times, mechanical properties, drug release, *in vivo* wound healing in rat and histopathology were assessed. The sodium fusidate-loaded FFH composed of sodium fusidate/PVP/PVA/propylene glycol/ethanol/water at the weight ratio of 1/2/12/3/8/74 could form a corresponding dried film in the wound sites promptly due to fast film-forming time of about 4 min. This FFH showed an appropriate hardness and adhesiveness. Furthermore, this corresponding dried film provided an excellent flexibility and elasticity, and gave relatively high drug release. As compared with the sodium fusidate-loaded commercial product, it significantly improved excision and infection wound healing in rats. This FFH was stable at 45 °C for at least 6 months. Therefore, this novel sodium fusidate-loaded FFH would be an effective pharmaceutical product with easy application for the treatment of wounds.

1. Introduction

Wound healing is a biological process by which the structure and function of damaged skin are restored to their normal skin. For desirable wound care, the wound dressing formulations must serve the followings; (a) provision of moisture and occlusion, (b) protection from pollution and infections, and (c) easy application and removal with the avoidance of dressing-related trauma (Singer and Dagum, 2008; Thu et al., 2012). Various types of wound dressing formulations prepared with many hydrophilic polymers, such as polyvinylalcohol (PVA), chitosan, sodium carboxymethyl cellulose and sodium alginate, have been developed, leading to their excellent efficacy in wound care (Choi et al., 2014; Jin et al., 2015a; Kim et al., 2008a; Lim et al., 2010; Lee et al., 2010). However, wound dressing formulations have demerits such as the complicated manufacturing process and difficult administration (Hwang et al., 2010; Kim et al., 2008b). The film-forming hydrogel (FFH) is a hydrogel dosage form which transform from the hydrogel to film-type by solvent evaporation after application to the wound site (Davis et al., 2001; Schuren et al., 2005). This formulation has the advantages of both hydrogel and film types. Compared with wound dressing forms, it offers easier use and application, and simpler manufacture. Furthermore, the FFH system can be freely applied to any wound site, even though the wound is curved and shaped. However, it is difficult to apply the big wound site because it has relatively low swelling capacity such as fluid uptake ability and water vapour transmission rate (De Cicco et al., 2014; Eaglstein et al., 2002). The FFH formulations, which were prepared using excess amounts of ethanol and acetone as solvents, and carbopol, octyl-2-cyanoacrylate, and Eudragit as film-forming polymers, have been studied as a transdermal pharmaceutical preparation for the sustained release of drugs (Asasutjarit et al., 2014; Sutar et al., 2008). The use of excess organic solvents might induce any irritations and damage to the skin (Asasutjarit et al., 2014; Davis et al., 2001). Furthermore, the FFH system was hardly attempted to be developed as a pharmaceutical product for wound healing.

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Therefore, in this study, in order to develop a novel sodium fusidate-loaded film-forming hydrogel (FFH) for effective wound healing, various FFH formulations and corresponding FFH dried films were prepared with drugs, PVA, polyvinylpyrrolidone (PVP), propylene glycol, water and small amounts of ethanol, and their film-forming times, mechanical properties, drug release, *in vivo* wound healing in rats, and histopathology were assessed. Here, sodium fusidate was used as a model drug. This drug is widely used as an antibiotic in the treatment of wound infections in human subjects, because it is effective against *Staphylococcus* and other Gram positive bacteria (Bonamonte et al., 2014; Gurel et al., 2015; Lim et al., 2010). Additionally, the sodium fusidate-loaded commercial ointment widely used for wound healing was frequently employed as a control model in the study on wound healing evaluation (Lee et al., 2010; Lim et al., 2010).

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (PVA, typical average Mw = 146,000–186,000; +99% hydrolysed) and polyvinyl pyrrolidone (PVP; K30) used as film formers in this study were provided by Sigma–Aldrich Co. (St. Louis, MO, USA) and BASF (Ludwigshafen, Germany), respectively. Polyethylene glycol (PEG; 600, 1500, 4000) and propylene glycol used as plasticizers were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Sodium fusidate was purchased from Novachem Co. (Wuhan, China). The commercial product (Fusidin™; Ointment type) was purchased from Dongwha Pharm. Co. (Seoul, South Korea). All other chemicals were used without any further purification.

2.2. Film-forming time and mechanical properties of FFHs

Preparation of FFHs: Sodium fusidate, various polymers and plasticisers were entirely dissolved in the mixture of ethanol and water using a mechanical stirrer, leading to the production of FFHs. Prior to experiments, the FFHs were kept in glass vials which were sealed tightly.

Film-forming time: Each FFH (0.2 g) was put onto the acrylic plate (2 cm × 2 cm) at 25 °C, and its film-forming degree was determined by the naked eye. All observations were assessed five times.

Hardness and adhesiveness: The mechanical properties of the FFHs were determined through Texture profile analysis (TPA) using the texture analyser (TA.XT2; Haslemere, Surrey, UK) with a 5 kg-loaded cell. Thirty grams of the FFH formulation was placed in a 100 ml beaker and sonicated in the ultrasonic water bath (B5510, Branson; Danbury, CT, USA) for 45 min to remove air bubbles. In TPA, the Perspex probe with a diameter of 10 mm was compressed into each sample at a defined rate of 2 mm/s to a depth of 15 mm. Five replicate analyses were carried out for each sample. From the resultant force-time plot (data not shown), the hardness and adhesiveness of the FFH were determined as the maximum force and the second area, respectively (Murphy et al., 2011; Hurler and Skalko-Basnet, 2012).

2.3. Mechanical properties of FFH dried films

Preparation of FFH dried films: The FFHs (0.2 g) were placed onto an acrylic plate (2 cm × 2 cm) and then kept at room temperature for 10 min in order to prepare the FFH dried films entirely.

Tensile strength and elongation at break: A texture analyser (TA.XT2; Haslemere, Surrey, UK) was employed for determining the tensile strength and elongation at break of the dried films (2 cm × 2 cm), according to ASTM D882 (Sabetzadeh et al., 2015). The tensile strength was determined by the maximum weight loaded at the time of rupture of the dried films. Furthermore, the elongation at break (%) was calculated by comparing the initial length of the dried films and the length at breakage (Zurdo Schroeder et al., 2007).

2.4. Drug release from sodium fusidate-loaded FFH dried films

The test on the drug release from the sodium fusidate-loaded FFH dried films was performed using a dialysis instrument (Dialysis tester; Lab Fine, Anyang, South Korea). The sodium fusidate-loaded FFH dried films were put into 50 ml of dissolution medium (phosphate buffer, pH 7.4) at 37 °C for 24 h. The stirring rate was 50 rpm. At pre-determined intervals, 0.5 ml of the sample was taken, diluted and filtered through syringe filter (0.45 μm, No. 6789-1304; Whatmann Co., Shrewsbury, MA, USA). The concentrations of sodium fusidate were checked using a HPLC system equipped with a separation module and photodiode array detector (Waters 2795; Waters Co., Milford, MA, USA) and consisting of an Inertsil ODS-2C18 column (5 μm, 15 cm × 4.6 mm; GL Sciences Inc., Tokyo, Japan). The mobile phase was composed of acetonitrile/sodium acetate buffer (pH 4.6) (75:25, volume ratio). The injection volume was 50 μl, the flow rate was 1 ml/min and the eluent was monitored at 365 nm. The inter-day and intra-day precision (0.055–0.721%) and accuracy (98.7–103.2%) were within the acceptable limits (r^2 = 0.999).

2.5. In vivo wound healing

In this *in vivo* test, male Sprague–Dawley rats weighting 250–280 g were purchased from Nara Biotech (Seoul, South Korea). The protocols for the animal studies were implemented consistent with the NIH Policy and Animal Welfare Act under approval by the Institutional Animal Care and Use Committee (IACUC) at Hanyang University. To create the diabetes-induced rats, streptozotocin (Sigma–Aldrich, St. Louis, MO, USA) in saline-sodium citrate buffer (pH 4.5) was intravenously administered to the rats at a dose of 60 mg/kg, followed by taking the blood from the tail vein of rats after 5 days and assessing the glucose titre *via* a blood glucose meter (Accu-chek®; Roche Diagnotics Korea Co., Seoul, South Korea). The diabetes-induced rats were anaesthetised by i.p. injection of Zoletil 50® (tiletamine/zolazepam). The dorsal hair of these animals was shaved with an electric razor.

At each trimmed area, a full thickness wound was made by excising a portion of the dorsal skin (1.5 × 1.5 cm^2) of each rat and sterilised with 70% ethanol, leading to producing the excision wounds. Moreover, a full thickness wound (1.0 × 1.0 cm^2) was inflicted at each trimmed portion on the rat skin, and then an aliquot of 50 μl of *Staphylococcus aureus* solution (KCCM 40050, 3.2×10^8/ml; American Type Collection Culture, Manassas, VA, USA) was poured onto this wound, leading to inducing the infection wounds. Each wound was covered with sterile gauze (control), the sodium fusidate-loaded FFH and the commercial product, respectively. In particular, the wound was covered and then fixed with an elastic adhesive bandage (Soft cloth tape®, 3 M, USA). All rats were separately kept in individual cages. At the predetermined intervals, each wound size was measured using a digital camera and surveyed using the Adobe® Acrobat® 7 Program (Jin et al., 2015a). The relative wound size reduction was calculated as follows (Jin et al., 2015b; Kim et al., 2008b). Relative wound size reduction (%) = $[(S_o - S_t)/S_o] \times 100$, where S_o and S_t are the wound size at initial time and time 't', respectively.