Wound Healing Evaluation of Sodium Fucidate-loaded Polyvinylalcohol/sodium Carboxymethylcellulose-based Wound Dressing

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(Received February 21, 2010/Revised April 13, 2010/Accepted April 20, 2010)

INTRODUCTION

In recent years, much attention has been focused on the research and development of polymer hydrogels for biomaterials, such as wound dressing and drug delivery systems. Rapid and proper healing is important in the treatment of wounds such as severe burns, trauma, diabetic, decubitus and venous stasis ulcers, and similar tissue damage.

The ideal wound dressing maintains a moist environment around the wound and absorbs the exudates from the wound surface (Turner, 1979). Acute and partial thickness wounds showed a significant increase in re-epithelialisation rates when they were maintained in a moist local environment (Winter, 1962; Hinnman and Maibach, 1963). Hydrogels, three-dimensional cross-linked hydrophilic polymers with a very high intrinsic content of water, can provide a moist environment to the wound area and absorb the exudates (Hoffman, 2002; Stashak et al., 2004; Valenta and Auner, 2004; Morin and Tomaselli, 2007; Ajji et al., 2008).

In our previous reports, nitrofurazone-loaded PVA/sodium alginate hydrogels (Kim et al., 2008a) and clindamycin-loaded PVA/sodium alginate hydrogels (Kim et al., 2008b) could not improved the healing effect compared to conventional products. Furthermore, instead of sodium alginate and other drugs, recent report showed that the novel sodium fucidate-loaded wound dressing, cross-linked hydrogel films were...
prepared with polyvinyl alcohol (PVA) and sodium carboxymethylcellulose (Na-CMC) using freeze-thawing method. These sodium fucidate-loaded PVA/Na-CMC-based wound dressings gave good forming and excellent gel property. However, the previous studies focused on investigating their preparation and gel characterisation, there has been lack of information on its wound healing effect. Thus, in this study, their in vivo wound healing test and histopathology were performed compared with the conventional ointment product. PVA is a semicrystalline co-polymer of vinyl acetate and vinyl alcohol that has been widely utilized in the chemical and medical industries because of its good properties like biocompatibility, non-toxicity, hydrophilicity, fiber/film forming ability, chemical resistance and protein absorption (Cascone et al., 1995). Furthermore, Na-CMC, a cellulose derivative, has been used in wound dressing since it is good biocompatible, hydrophilic, non-toxic and non-allergenic (Kim et al., 2006; Gaisford et al., 2009). Sodium fucidate with a steroid structure has been used topically in the treatment of skin and soft tissue infection (Dennis, 1999). It has a bacteriostatic effect at normal levels and a bactericidal effect at high levels. It exerts its effects by inhibiting ribosomal protein synthesis by bacteria. Sodium fucidate is especially effective against Gram-positive bacteria. There is no cross-resistance between Sodium fucidate and other antibiotics (Tabbara et al., 1989).

MATERIALS AND METHODS

Materials

PVA (typical average MW = 146,000-186,000; +99% hydrolysed) and Na-CMC (typical average MW = ~90,000) were purchased from Sigma-Aldrich Co. and Duksan Pure Chemical Co., respectively. Sodium fucidate and conventional product (Fucidin™; in an ointment form) were purchased from Dong-Hwa Pharmaceutical Co. All other chemicals were used without any further purification.

Preparation of hydrogels

PVA/Na-CMC hydrogels were obtained by the freezing-thawing (F-T) cycle. In brief, the solutions containing 10% w/v PVA (5 mL), 1.5% w/v Na-CMC (15 mL) and sodium fucidate (0 or 40 mg) were mixed by vortexing for 1 h and poured into Petri dishes. They were then frozen at -20°C for 18 h and then thawed at room temperature for 6 h for three consecutive cycles (Cascone et al., 1995; Yeo et al., 2000).

In vivo wound healing test

Male SD rats weighting approximately 250-280 g were used to evaluate the in vivo wound healing test of hydrogels. All animal care and procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989 and revised in 1999 by the Society of Toxicology (SOT, 1999). Furthermore, the protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of Yeungnam University. The rats were anaesthetised by i.p. injection of Zoletil 50® (tiletamine/zolazepam) and the dorsal hair of each animal was shaved with an electric razor. After creating two full thickness wound areas (1.5 × 1.5 cm) by excising the dorsum, 70% ethanol was used for sterilisation. Each wound was covered with sterile gauze (control), the hydrogel without drug, the hydrogel with drug and the commercial product, respectively. The hydrogel without or with drug was composed of 2.5% PVA, 1.125% Na-CMC and no drug, or 0.2% of drug, respectively. All materials were fixed with an elastic adhesive bandage. All rats were separately kept in individual cages. At the 3rd, 6th, 9th, 12th and 15th days after the operation, each wound size was measured using a digital camera (Kim et al., 2008b).

Histopathology

Histological process: The wounded area of skin containing dermis and hypodermis was sampled and crossly trimmed. All trimmed skins were fixed in 10% neutral buffered formalin. After paraffin embedding, 3-4 μm sections were prepared. Representative sections were stained with hematoxylin and eosin (H&E) for microscopic examination, or Masson’s trichrome for collagen fibres (Kim et al., 2000).

Histomorphometry: The desquamated epithelium regions (nm), numbers of microvessels in granulation tissues (vessels/mm² of field), numbers of infiltrated inflammatory cells in granulation tissues (cells/mm² of field), percentages of collagen-occupied regions in granulation tissues (%/mm² of field) and thicknesses of central regions of granulation tissues (mm from epidermis to dermis) were measured on the histological skin samples using a digital image analyser (DMI-300, DMI), respectively (Quintanilha Ribeiro et al., 2008).

Statistical analysis: Multiple comparison tests for different dose groups were conducted. Variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from variance homogeneity, the data were analysed by one way ANOVA test and the least significant differences (LSD) multi-comparison test. In case of significant deviations from the variance, homogeneity was observed using the Levene test, a non-parametric comparison