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Research paper

Development and characterization of thermo-sensitive films containing asiaticoside based on polyvinyl alcohol and Methylcellulose

Heyang Zhang, Yuwen Xie, Yue Tang*, Suhui Ni, Bo Wang, Zhen Chen, Xuejing Liu

School of Pharmacy, China Pharmaceutical University, Nanjing 211198, PR China

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ABSTRACT

This study aims at developing thermo-sensitive films and exploring their properties, especially as an asiaticoside-loaded wound dressing. The films were prepared from polyvinyl alcohol and methylcellulose by the casting method. The properties of films including mechanical properties, water vapor permeability and water solubility were evaluated based on the single factor design and the orthogonal design to optimize the formulation of thermo-sensitive films. Others, such as in vitro release, stability, skin irritation and in vivo wound healing effect were also investigated. The thermo-sensitive films mainly composited polyvinyl alcohol and methylcellulose were prepared within the range of temperature from 32 to 35 °C in 4 min. They were transparent, nonirritating, water-proof and biodegradable. A sustained-release pattern of the films was observed in vitro by the Franz diffusion cell. The accelerated stability test has illustrated that the formulation remained stable for 6 months. From the macroscopic and histological examination, it was observed that the asiaticoside-loaded films had better healing effect on the wounds than the control formulation (normal saline), suggesting the asiaticoside-loaded films were effective to promote wounds healing, reduce the administered frequency, improve the biomedical efficacy and enhance the patient acceptability both from economic and aesthetic perspectives.

1. Introduction

Wound healing is a complex biological process including hemostasis, inflammation, proliferation and maturation. An effective wound dressing should promote the various stages of wound healing and create better cosmetic results. Based on the types of wounds, various formulations were developed such as spray and aerosol to develop more effective wound dressing for skin wounds. The films prepared from polyvinyl alcohol (PVA) and methylcellulose (MC) with the aids of physical and chemical methods in our laboratory were temperature-responsive to the range of temperature from 32 to 35 °C. Specifically, the thermo-sensitive films can be formed in a short period after the solution was sprayed on the skin as the temperature of skin is about 32–35 °C. The resultant films can be developed as drug carriers especially for healing skin wounds as they can provide some desirable performances including avoidance of secondary trauma to the neo-tissues, reduction of skin irritation, absorb and prevent loss of body fluids, barrier against bacteria, good handling, bio-compatibility, non-toxicity, etc [1].

PVA (as shown in Fig. 1) is a hydrophilic, biodegradable and biocompatible synthetic polymer which is mainly composed of C–C and hydroxyl groups. Due to the numerous favorable characteristics, specifically the excellent film-forming properties, easy replacement, transparency to allow healing follow up, control of drug dosage, etc [2], films derived from PVA recently have been widely used in biomedical applications [3–5], such as wound dressing, artificial skin and drug delivery devices [6]. It is already known that films prepared from PVA with a relatively high degree of hydrolysis have a nature of being insoluble in water at a temperature below 40 °C. The useful mechanical strength of the produced film depends on the polymerization degree of PVA which are preferably polymerized to more than 700, or more preferably more than 900. PVA with a polymerization degree of more than 900 can produce a film having sufficient mechanical strength. The hydrolysis degree of the PVA substantially influences the water solubility of the resultant film. Films insoluble in water at the temperature below 40 °C can be prepared from PVA with a hydrolysis degree

* Corresponding author. China Pharmaceutical University, No. 639, Longmian Avenue, Nanjing 211198, PR China.
E-mail addresses: tangue@cpu.edu.cn, tycpu2008@sina.com (Y. Tang).

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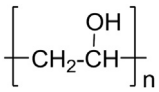

Fig. 1. The chemical structure of PVA.

exceeding 97% while the resultant film prepared from PVA with a hydrolysis degree below 97% will have an undesirable nature of being readily soluble in water at a temperature of even less than 40 °C [7]. As the physiological temperature of skin was 32–35 °C, no interference can be found with the film-forming and solubility in water of PVA by the skin temperature. Such nature makes the film available for use as a wound dressing. However, films prepared from PVA are no thermo-sensitive although PVA hydrogels have been paid considerable attention in wounds management [8,9].

Methylcellulose (MC) as shown in Fig. 2 is well-known for the thermo-reversibility that form hydrogels in water upon heating and subsequently dissolve upon cooling [10,11]. The gelation of MC depends on the temperature [11,12] and the type of solvent [13]. The effect of salts on the gelation temperature of MC has been studied extensively. As reported that the salt is capable of reducing the gelation temperature of the MC solution [13–17] and anion in a salt, believed to deliver stronger effect than cation [15,18,19]. A typical Hofmeister order for anions was reported as $SO_4^{2-} > F^- > Cl^- > Br^- > NO_3^- > ClO_4^- > I^- > SCN^-$ [20]. It was found that PVA was used to reduce the gelation temperature of MC and the gelation temperature of the MC decreased further up to physiological temperature as the molecular weight of PVA increased.

Asiaticoside (AC) is an active component derived from the leaves of *Centella asiatica* (L.) (as shown in Fig. 3) and displays healing activity of wounds as evidenced by the observed increase in antioxidant levels at an initial stage of healing of excision-type cutaneous wounds in rats [21]. It also can inhibit proliferative activity related to keloid and hypertrophic scar, stimulate extracellular matrix accumulation in rat wounds [22,23], increase the migration rates and initial attachment of skin cells and promote normal human dermal fibroblast proliferation. The biological activities of AC support its use as a promoter of wound healing [24].

In this study, AC was loaded in the thermo-sensitive films and used for healing skin wounds. The mechanical properties mainly including tensile strength, tensile elongation and elastic modulus were also studied. Other properties, such as water vapor permeability, water solubility, surface morphology, stability, in vitro release and in vivo test were also investigated. The skin irritation tests on healthy and injured skins were evaluated when compared with the control group that treated with normal saline. Similarly, comparisons were made against the epidermal growth factor (GeneTime®) and controlled group (normal saline) to study their healing activity according to the ulcer index, macroscopic and histological examination.

2. Materials and methods

2.1. Materials

PVA was purchased from Sinopharm Chemical Reagent Co. Ltd

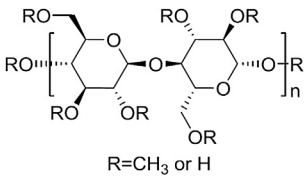

Fig. 2. The chemical structure of MC.

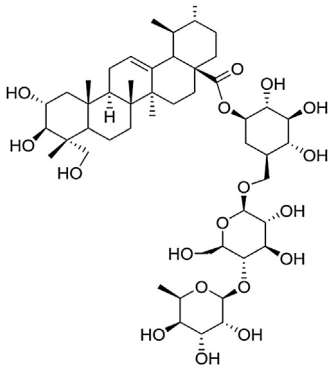

Fig. 3. The chemical structure of AC.

(Shanghai, China). The average degree of polymerization and hydrolysis of PVA were 2400–2500 and 98–99%, respectively. Methylcellulose (MC, MetoloseSM-400) was provided by Colorcon (Shanghai, China). Asiaticoside (purity >90%) was obtained from Changzhou Natural Pharmaceutical Co. Ltd (Guangxi, China). The standard of asiaticoside (purity >98%) was purchased from Chinese CRM/RM Information Center (Beijing, China). Methanol and acetic acid were of HPLC grade and obtained from Hanbang Science and Technology Co. Ltd (Jiangsu, China). GeneTime® (15 ml, 2000 IU/ml) was provided by Watsin Genetech Co. Ltd (Shenzhen, China). All other chemicals used were of analytical grade and purchased from Nanjing Chemical Reagent Co. Ltd (Nanjing, China) and used without further purification.

2.2. Methods

2.2.1. Films preparation

The films were prepared by casting method [25]. Firstly, a known amount of PVA was dissolved in deionized water at about 70 °C to prepare 7.5wt% solutions for swelling and then heated up to 90 °C to obtain well-proportioned solution. Secondly, MC (2.0 g) was dissolved in deionized water (100 ml) and stored at 4 °C for at least 12 h and no deposits were observed. Glycerol (GLY) and sodium chloride (NaCl) were blended in deionized water at room temperature. Then AC, GLY and NaCl were added to the mixed solution (PVA/MC = 2.7/4, v/v) dropwise to form homogenous solution in ice bath with vigorous stirring by magnetic stirrer. Finally, the solution prepared above was cast on framed glass plates with 5 ml/50 cm^2 and then dried at approximate 33 °C to obtain various films. Meanwhile, the time of films-forming was investigated before and after addition of AC into the mixed solution with thermo-sensitivity, i.e. blank films and AC-loaded films, respectively. The average time was calculated by three films.

2.3. Film characterization

2.3.1. FTIR analysis

Chemical structure changes in PVA, MC and GLY were confirmed with a Fourier transform infrared spectrophotometer (FT-IR) (Tensor 27, Bruck Co. Ltd, Germany). After drying in the vacuum oven at 60 °C overnight, samples were prepared in the form of KBr pellets. The spectra were recorded over the wave number from 400 cm^{-1} to 4000 cm^{-1}, operating with a 4 cm^{-1} resolution.

2.3.2. Optical microscope and visual aspect

Surface morphology of the films that prepared with various concentration of PVA, PVA/MC, PVA/GLY as well as blank films and AC-loaded films was observed by naked eye and ECLIPSE Ti–S