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Development and physicochemical characterization of alginate composite film loaded with simvastatin as a potential wound dressing

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ABSTRACT

Previously, studies have demonstrated that topical application of simvastatin can promote wound healing in diabetic mice via augmentation of angiogenesis and lymphangiogenesis. This study aimed to formulate and characterize simvastatin in alginate-based composite film wound dressings. Biopolymers used for composite films were sodium alginate blended with pectin or gelatin. The films were prepared and characterized based on their physical properties, surface morphology, mechanical strength and rheology. Then, in vitro drug releases from the films were investigated and, finally, the cell viability assay was performed to assess the cytotoxicity profile. From the pre-formulation studies, alginate/pectin composite film showed to possess desirable wound dressing properties and superior mechanical properties. The in vitro drug release profile revealed that alginate/pectin film produced a controlled release drug profile, and cell viability assay showed that the film was non-toxic. In summary, alginate/pectin composite film is suitable to be formulated with simvastatin as a potential wound dressing.

1. Introduction

Wound dressing has a predominant function in wound management. Chronic wounds, such as diabetic leg ulcers, are long lasting wounds that do not undergo the gradual process of healing and instead remain in the inflammatory phase of management. Medicated dressing by impregnating a drug of different pharmacological actions is an effective approach for achieving a quick and optimum healing response (Pawar, Tetteh & Boateng, 2013).

Simvastatin, an inhibitor of HMG-CoA reductase, is conventionally used for lipid lowering effects in cardiovascular diseases. Recently, its therapeutic effects beyond plasma cholesterol lowering in cell regeneration, such as the induction of bone tissue regeneration (Bae et al., 2011; Tanigo, Takaoka, & Tabata, 2010), anti-inflammatory activity on skin (Adami et al., 2012) and fracture healing (Fukui et al., 2012), have been demonstrated. Asai et al. (2012) successfully demonstrated that the topical application of simvastatin promotes wound healing in diabetic mice by augmenting angiogenesis and lymphangiogenesis. The statins appear to protect against ischemic injury and stimulate angiogenesis. This angiogenic effect is partially mediated by direct regulation of the proliferation of endothelial cells and capillary morphogenesis via the PI3-kinase/Akt pathway (Kureishi et al., 2000). Simvastatin also promotes capillary morphogenesis in vitro and exerts an anti-apoptotic effect on lymphatic endothelial cells, which suggests the lymphangiogenic effects of simvastatin in wound healing (Asai et al., 2012). Currently, there is a lack of study in the literature with respect to developing a wound delivery system containing simvastatin for the chronic wound healing application.

In the wound care market, a plethora of wound dressings, ranging from conventional gauze to modern dressings, are available. Moisture-retentive dressings have been preferred in chronic wound management, owing to their ability to provide a moist environment, which is essential for effective chronic wound healing. Film dressings are known to be one of the most popular choices. Film dressings are simple, thin and semipermeable for effective water vapor and oxygen exchange. When in contact with wound exudates, the film transforms into a gel, creating a moist environment around the wound area. Moreover, the pliable feature of a film dressing is useful for application on flexible body areas such as joints (Moura, Dias, Carvalho, & de Sousa, 2013).

Natural biopolymers such as alginate, pectin and gelatin have been widely investigated as materials for wound dressings (Mogoşanu & Grumezescu, 2014). Among these biopolymers, alginate dressings are the most commonly used in different stages

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of wound healing due to alginate being a biocompatible and biodegradable polymer (Lee & Mooney, 2012) that possesses good film forming characteristics (Wang, Liu, Holmes, Kerry, & Kerry, 2007). Moreover, it can trigger macrophage activity and increases cytokine levels in wounds (Yang & Jones, 2009). Pectin is a complex polysaccharide consisting mainly of esterified D-galacturonic acid. It is found in the most primary cell walls of terrestrial plants and is also one of the major constituents in citrus fruit by-products. Pectin offers several advantages in wound healing, such as (1) its hydrophilicity which permits the removal of exudates by forming a soft gel over the wound bed, (2) the retention of an acid environment during pectin solubilization which may act as bacterial or viral barrier and (3) its ability as binding agent for protecting growth factors, which responsible for new cell generation, from degradation (Munarin, Tanzi, & Petrini, 2012).

Gelatin is a peptide produced by the hydrolysis of collagen extracted mainly from animal sources and is widely used in food and pharmaceutical applications due to its biocompatibility, safety and biodegradability (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). It also gives prompt hemostasis, prevents wound contracture and aides in cell adhesion and proliferation during wound healing process (Boateng, Burgos-Amador, Okeke, & Pawar, 2015).

Previous research has suggested that composite film is more superior to single polymeric film and is a simple approach for enhancing the mechanical properties of films (Han, Yan, Chen, & Li, 2011; Thu, Zulfakar, & Ng, 2012). The scope of the present work was to develop simvastatin-loaded film wound dressings composed of alginate, pectin and gelatin. The films were prepared using the solvent cast technique and characterized based on their physical properties, surface morphology, mechanical strength and rheology. Then, in vitro drug releases from the films were investigated and, finally, the cell viability on human fibroblast cells of the films was carried out to assess their cytotoxicity profiles.

2. Materials and methods

2.1. Materials

Sodium alginate (CAS No. 9005-38-3, $M_W \sim 20$–40 kDa) and gelatin (CAS No. 9000-70-8, Ph. Eur grade, type B) were obtained from Sigma-Aldrich (UK). Simvastatin (CAS No. 79902-63-9, pharmaceutical secondary standard) was supplied by Sigma-Aldrich (USA). High methoxy pectin pure (CAS No. 9000-69-5, $M_W \sim 30{,}000$–$100{,}000$) was purchased from R&M Chemicals (UK). Glycerol (≥99.5%) was procured from Sigma-Aldrich (Germany). Calcium chloride dehydrate and ethanol were obtained from Merck (Germany). All other chemicals were of analytical grade, and distilled water was used throughout.

2.2. Preparation of gels and films

Aqueous gel (blank) and drug-loaded gels were prepared using the solvent casting method. Five percent (5% w/w) sodium alginate (SA) and SA composite films blended with pectin (SA–PC) or gelatin (SA–GL) of equal mass fractions (1:1) were prepared. The compositions of gels for film casting are presented in Table 1. The gels for casting films were prepared by dissolving the polymers in distilled water containing glycerol with a constant stirring at 1000 rpm for 2–3 h at 40 °C. These homogenized gels were sonicated for at least 1 h and were then left to stand at room temperature until all remaining air bubbles were eliminated. The blank films were dried cast by pouring the gel (20 g) into a plastic Petri dish (d = 90 mm) and then drying in oven at 45 °C for 48 h. The drug-loaded polymeric gels were prepared by incorporating 5 mL of ethanolic solution of SIM (20 mg/mL) into the gels to attain a drug concentration of 2% w/w in gels. These drug-loaded gels were dried in an oven as described earlier for blank gels and eventually stored in a desiccator.

Table 1
Composition of the alginate and composite film dressings containing simvastatin.

Film composition*	SA	SA–SIM	SA–PC	SA–PC–SIM	SA–GL	SA–GL–SIM
Sodium alginate (g)	5	5	2.5	2.5	2.5	2.5
Pectin (g)	–	–	2.5	2.5	–	–
Gelatin (g)	–	–	–	–	2.5	2.5
Glycerin (g)	2.5	2.5	2.5	2.5	2.5	2.5
Simvastatin (g)	–	0.1	–	0.1	–	0.1
Ethanol (mL)	–	5	–	5	–	5
Distilled water (mL)	92.5	87.4	92.5	87.4	92.5	87.4

* Abbreviation: SA—sodium alginate, PC—pectin, GL—gelatin, SIM—simvastatin.

2.3. Film thickness and mechanical properties

The thickness of films was measured by using a digital caliper (Digimatic Micrometer MDC-S, Mitutoyo Co., Japan, sensitivity = 0.001 mm) at five positions (one at the center, four near the edges). This test was carried out in triplicate for each film and mean values were recorded. Tensile strength (TS) and percentage elongation at break (%E) of the films were determined using the Instron Universal Testing Machine (Model 5567, USA) according to the ASTM D 882-02 standard (ASTM, 2002). The sample specimens were conditioned at 25 °C and 50 ± 5% RH for 48 h prior to analysis. Films were cut to dumbbell-shaped strips that were 30 mm long and 5 mm wide using an ASTM standard dumbbell shape template and a cutter press (GT-7016-A Gotech Testing Machine, Malaysia). The mechanical properties of specimens were measured by stretching the films at a crosshead speed of 5 mm/min to their breaking point. At least five replicates from each type of film were used for this analysis.

The tensile strength (N/mm^2) and elongation at break (%E) were calculated based on the following equations:

$$\mathrm{TS} = \frac{\text{Maximum load at break}}{\text{Transverse section area}} \quad (1)$$

$$\%E = \frac{\text{Extension of length at rupture}}{\text{Initial length}} \times 100 \quad (2)$$

2.4. Water vapor transmission

A procedure for water vapor transmission (WVT) of the film over 24 h was adopted based on the modification of the previously described method (Thu et al., 2012). Films were cut into a disc-like shape. Five grams of calcium chloride as a desiccant was added to a dry glass vial, and films of defined diameter (exposed diameter = 1.1 cm) were sealed to the cap using a rubber ring and then mounted onto the brim of a vial. The vials were weighed and put into a humidity chamber (Terchy HRM 80 FA, Taiwan) at constant relative humidity (84%) and temperature (25 °C). The presence of a fan in the chamber ensured a uniform RH throughout the experiment. Gain in the weight of the vial was recorded every 1 h for a period of 8 h and then at 24 and 48 h. Triplicated measurements was run, and mean values were obtained. WVT over 24 h was determined using the following equation:

$$\mathrm{WVT} = \frac{W}{S} \quad (3)$$

where W is the gain in weight of the desiccant over 24 h and S is the exposed surface area of the film (m^2), WVT (g/m^2/day).