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Composite alginate and gelatin based bio-polymeric wafers containing silver sulfadiazine for wound healing

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ABSTRACT

Lyophilized wafers comprising sodium alginate (SA) and gelatin (GE) (0/100, 75/25, 50/50, 25/75, 0/100 SA/GE, respectively) with silver sulfadiazine (SSD, 0.1% w/w) have been developed for potential application on infected chronic wounds. Polymer–drug interactions and physical form were characterized by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD), respectively, while morphological structure was examined using scanning electron microscopy (SEM). Functional characteristics [(mechanical hardness and adhesion using texture analyzer, and swelling capacity)] of blank wafers were determined as performed in order to select the optimal formulations for drug loading. Finally, the *in vitro* drug dissolution properties of two selected drug loaded wafers were investigated. There was an increase in hardness and a decrease in mucoadhesion with increasing GE content. FTIR showed hydrogen bonding and electrostatic interaction between carboxyl of SA and amide of GE but no interaction between the polymers and drug was observed, with XRD showing that SSD remained crystalline during gel formulation and freeze–drying. The results suggest that 75/25 SA/GE formulations are the ideal formulations due to their uniformity and optimal mucoadhesivity and hydration. The drug loaded wafers showed controlled release of SSD over a 7 h period which is expected to reduce bacterial load within infected wounds.

1. Introduction

In recent years, natural biopolymers such as alginate, collagen and chitosan have been studied because of their importance in formulation of different dressings for healing of burns and other types of wounds. This is due to several favorable characteristics including biocompatibility, biodegradability and some structural similarities with human tissues, as well as their implication in the repair of damaged tissues and consequently skin and tissue regeneration [1–3].

Alginate is an anionic polysaccharide, extracted from brown algae (Phaeophyceae) or obtained by bacterial biosynthesis from *Azotobacter* and *Pseudomonas* spp. It is composed of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues linked in homopolymeric blocks (—MMM— or —GGG—) or random blocks (—MGMG—). Depending on the block content, length and distribution in the polymeric chain, alginates possess different physical, chemical and gelling properties [4]. Alginate dressings are characterized by the formation of a gel due to the exchange between the ions present in the dressing and wound exudate [5]. This gel creates a moist environment that promotes healing and facilities easy removal [6]. This together with its high tissue compatibility, low toxicity and good mucoadhesive properties allow alginates to be used as biomaterials for wound dressings [7]. The impact of cross-linker cations such as Na^+, Ca^{2+}, Cu^{2+} or Zn^{2+} in modifying dressings' functional wound healing characteristics such as tensile strength and hydration has been reported [8]. However, with time, hydrated alginate can lose the cation cross-linkers, resulting in gel degradation. Therefore, it has been recommended to combine alginates with other biopolymers such as gelatin or chitosan in a single formulation [9].

Gelatin is a denatured protein from the triple helix of collagen. In solution, the chains are converted from random spirals at moderate temperature to helices once the temperature decreases below ambient, thus behaving as a gel [10]. Ideal characteristics such as biodegradability, ease of processing and its antigenic activity in physiological environments have resulted in the wide use of gelatin in biomedical applications. It also provides hemostasis and facilitates cell adhesion and proliferation during wound healing [11]. However, poor mechanical properties and low thermal stability

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have been described as some of the disadvantages of this biomaterial which can be improved by cross-linking and/or combining with other polymers [12]. Balakrishna and co-workers developed a hydrogel dressing based on the beneficial properties of oxidized alginate, gelatin and borax with the purpose of making a potential dressing that maintains a moist wound environment [13]. It has also been reported that oxidized alginate could be successfully utilized to stabilize gelatin films and therefore improve their mechanical properties [14].

Metal antimicrobials have been used over the years to combat bacterial infection with silver being the most common metal based antimicrobial in medicated wound dressings. At an appropriate concentration, silver shows broad spectrum activity against fungal and bacteria cells including methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococci* (VRE) [15]. Different products have been developed with silver, such as foams (Contreet F®), hydrocolloids (Contreet H®), alginates (Anticoat absorbent®) and films (Arglaes®) with indications for burns and heavily colonized wounds [16,17]. However, these products are all single polymer matrix systems which do not always control drug release appropriately as well as exhibit optimal functional properties such as adhesion, swelling and mechanical strength. The use of composite dressings, combining more than one polymer with enhanced physical–mechanical characteristics has gained recent interest [2,3].

The aim of this study therefore, was to formulate freeze-dried wafers from gels combining different ratios of sodium alginate (SA) and gelatin (GE) and loaded with silver sulphadiazine (SSD) for potential application to infected wounds. Different analytical techniques have been used to characterize the functional physico-chemical properties of the starting polymers and wafer formulations, including scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), as well as texture analysis ('hardness', and adhesion), swelling and *in vitro* drug dissolution studies.

2. Experimental

2.1. Materials

Silver sulfadiazine [SSD, (batch number: 48118156)], Pluronic [F68, (batch number: 020M0029)], calcium chloride (batch number: 1291383) and tris methylamine (batch number: 87203010) were obtained from Sigma-Aldrich (Steinnheim, Germany). Sodium alginate [SA, (batch number: 0804532)] and sodium chloride (batch number: 1095753), were purchased from Fisher Scientific (Leicestershire, UK). Gelatin [GE, (batch number: 54008P03)] was obtained from Fluka Analytical (Steinheim, Germany).

2.2. Preparation of gels and freeze-dried wafers

Blank (BL) and SSD loaded (DL) gels were prepared with varying concentrations of SA and GE while keeping the amount of the other components (SSD and pluronic acid—F68) constant (Table 1).

The gels of SA and GE were prepared by dispersing the polymers and surfactant (F-68) in hot distilled water (50 °C) with continuous stirring until they were completely dissolved. For gels containing SSD, the drug was first dispersed into the vortex of hot surfactant solution (50 °C) before adding the different polymers (SA and GE). 7 g each of gel was transferred into 6 well plates (diameter 35 mm) and lyophilized using a Virtis Advantage XL 70 freeze dryer (Biopharma Process System, Winchester, UK) in automatic mode. The lyophilization procedure involved freezing the gels in a series of thermal ramps to −50 °C over 7 h (freezing phase), then heating during the primary drying phase to sublimate the ice under vacuum at −15 °C (24 h), followed by secondary heating at 20 °C for 7 h.

Table 1
Composition of blank (BL) and drug loaded (DL) loaded gels with total polymer (SA and GE) content of 3% w/w in each case.

% ratio of SA/GE	SA (% w/w)	GE (% w/w)	F68 (% w/w)	Drug (% w/w)
BL 100/0	3.00	0.00	0.20	–
BL 75/25	2.25	0.75	0.20	–
BL 50/50	1.50	1.50	0.20	–
BL 25/75	0.75	2.25	0.20	–
BL 0/100	0.00	3.00	0.20	–
DL 100/0	3.00	0.00	0.20	0.10 SSD
DL 75/25	2.25	0.75	0.20	0.10SSD
DL 50/50	1.50	1.50	0.20	0.10 SSD
DL 25/75	0.75	2.25	0.20	0.10SSD
DL 0/100	0.00	3.00	0.20	0.10 SSD

2.3. Visual evaluation and scanning electron microscopy (SEM)

The wafers were visually evaluated by capturing digital images of the different formulations. Further, the wafers were examined microscopically under low vacuum by a Jeol JSM-5310LV scanning microscope to obtain high-resolution surface information of their morphological structure. The samples were cut into small, thin pieces and placed on double-sided carbon tape on 15 mm aluminum stubs. Sample images were acquired at magnifications of ranging from ×50 to ×200.

2.4. X-ray diffraction (XRD)

A D8 Advance X-ray diffractometer (Bruker, Coventry, UK) equipped with Lynx Eye detector was employed to determine the crystalline or amorphous nature of the different pure starting materials and within the formulated wafers. The freeze-dried wafers were compressed using a pair of cover glasses to a size of 0.3 mm and introduced into the sample holder. All the samples were scanned between 2 theta of 5° and 45° with a step size of 0.02 and a scan speed of 0.4 s. The same process was repeated for SSD, SA, GE and F-68.

2.5. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy

FTIR spectra of wafers and the different starting materials were acquired on a FTIR spectrophotometer (Thermo Nicolet, Thermoscientific, UK) combined with ZnSe attenuated total reflectance (ATR) crystal accessory based on a previously reported method [18]. After the crystal area had been cleaned, the material was placed on the ATR crystal and pressed by a pressure clamp positioned over the crystal/sample area to allow optimal contact between the material and the ATR crystal. The spectra were collected at a resolution of 4 cm^{-1} over a range of 650 to 4000 cm^{-1}.

2.6. Swelling study

This test determined the maximum hydration capacity of the wafers in simulated wound fluid (SWF). The SWF contained 0.02 M calcium chloride, 0.4 M sodium chloride and 0.08 M tris methylamine in deionized water. To adjust the pH of the solution, 2 M of hydrochloric acid (HCl) was added until a pH of 7.5 was attained [19]. Samples ($n = 4$) were initially weighed and immersed into 20 ml of SWF at 37 °C. The change in weight of the wafers was measured every 15 min up to 120 min to observe the swelling behavior. At each time point, the hydrated wafers were carefully removed,