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Polyox and carrageenan based composite film dressing containing anti-microbial and anti-inflammatory drugs for effective wound healing

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ABSTRACT

Polyethylene oxide (Polyox) and carrageenan based solvent cast films have been formulated as dressings for drug delivery to wounds. Films plasticised with glycerol were loaded with streptomycin (30%, w/w) and diclofenac (10%, w/w) for enhanced healing effects in chronic wounds. Blank and drug loaded films were characterised by texture analysis (for mechanical and mucoadhesive properties), scanning electron microscopy, differential scanning calorimetry, X-ray diffraction and Fourier transform infrared spectroscopy. In addition, swelling, *in vitro* drug release and antibacterial studies were conducted to further characterise the films. Both blank and drug loaded films showed a smooth, homogeneous surface morphology, excellent transparency, high elasticity and acceptable tensile (mechanical) properties. The drug loaded films showed a high capacity to absorb simulated wound fluid and significant mucoadhesion force which is expected to allow effective adherence to and protection of the wound. The films showed controlled release of both streptomycin and diclofenac for 72 h. These drug loaded films produced higher zones of inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* compared to the individual drugs zones of inhibition. Incorporation of streptomycin can prevent and treat chronic wound infections whereas diclofenac can target the inflammatory phase of wound healing to relieve pain and swelling.

1. Introduction

A wound is defined as disruption of normal anatomic structure and function (Boateng et al., 2008) and represents damage of natural defence barriers which encourages invasion by microorganisms (Kumar and Leaper, 2005). Wound repair normally involves systematic, co-ordinated and balanced activity of inflammatory, vascular, connective tissue and epithelial cells (Boateng et al., 2008). The existence of a variety of wound types with diverse healing approaches has resulted in the introduction of different types of wound dressings for successful wound healing. Wound infection is one of the most significant factors that delay healing when microorganisms compete with the host immune system and subsequently invade viable tissue. Most wound infections involve aerobes (*Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*) and anaerobes (*Pseudomonas aeruginosa*, *Bacteroides fragilis*, *Peptostreptococcus* spp., *Clostridium* spp., *Prevotella* spp., and *Fusobacterium* spp.) (Brook and Frazier, 1998; Duerden, 1994). Many published literature references refer to a figure of 10^6 CFU/ml of wound fluid or 10^5 CFU/g of tissue as a criterion for infection (White et al., 2001). Chronic wounds associated with ulcers and diabetes mellitus are susceptible to infection (Bowler et al., 2001) and up to 75% of chronic burn injuries also involve some form of infection (Revathi et al., 1998).

Effective management of wound infection necessitates reduced exogenous microbial contamination (bio-burden), debridement of devitalised tissue, use of appropriate dressing and topical and systemic broad-spectrum antimicrobial agents, maximisation of immune resistance and provision of adequate nutrition (Bowler et al., 2001). Antimicrobial agents such as antiseptics have high specificity to treat wound infection and ultimately improve wound healing (Forbes, 1961). However, the emergence of microbial resistance has resulted in the need to find alternative treatments for wound infections. In addition, systemic antibiotic treatment can be difficult in certain ulcers such as diabetic ulcers due to the poor blood circulation at the extremities (Bowler et al., 2001). In modern wound care practice, antibiotics such as neomycin, bacitracin, streptomycin (STP), gentamycin and polymixin and/or combinations are used to treat chronic wounds (Bowler et al., 2001; Howes, 1947; Pielesz et al., 2011).

Abbreviations: ATR, attenuated total reflectance; BLK, blank; BSA, bovine serum albumin; CAR, carrageenan CFU-colony forming units; DLF, diclofenac; DL, drug loaded; DSC, differential scanning calorimetry; FTIR, Fourier transform infra red; F_{max}, maximum force; GLY, glycerol; POL, polyox; SEM, scanning electron microscopy; STP, streptomycin; SWF, simulated wound fluid; TA, texture analysis; XRD, X-ray diffraction; WOA, work of adhesion; ZOI, zone of inhibition.

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It has been reported that diclofenac sodium (DLF) possesses low to moderate antibacterial activity against Gram positive and Gram negative bacteria and systemic STP in combination with DLF demonstrated synergistic antimicrobial activity (Dutta et al., 2004; Mazumdar et al., 2009). Combination of STP and DLF within a single dressing is expected to act on two phases of wound healing, *i.e.* preventing as well as treating wound infections (STP) and target the inflammatory phase (DLF) to relieve pain and swelling associated with injury. Films are considered as simple and effective dressings for creating a moist wound environment to promote wound healing (Sussman, 2010). Films also have the advantage of ease of application due to their flexibility around the joints and other difficult areas and also avoid painful removal in burn wounds (Jurgens et al., 1995).

Combining biocompatible biopolymers with synthetic polymers is of particular significance as either on its own cannot always meet all the complex demands within a biological system (Çaykara et al., 2005; Jagadish and Raj, 2011). This reduces the disadvantages of each individual polymer whilst maximising the optimum properties of each in the resulting combined entity. It has been demonstrated that hydroxyl groups of cellulose and polysaccharides can form hydrogen bonds with the ether oxygen of polyethylene oxide (POL) (Çaykara et al., 2005; Jagadish and Raj, 2011; Kondo and Sawatari, 1994).

In this study, we report on solvent cast film dressings formulated by combining a synthetic polymer; Polyox^{TM} (POL) with a biomaterial; carrageenan (CAR) and loaded with two drugs, streptomycin (STP) and diclofenac (DLF). Films were prepared by the solvent casting approach from aqueous gels of the polymers and characterised for functional properties expected for wound dressings.

2. Experimental

2.1. Materials

(Polyox^{TM} WSR 301 ≈4000 kDa) was obtained as a gift from Colorcon Ltd. (Dartford, UK), κ-carrageenan (Gelcarin GP 812 NF) was obtained from IMCD Ltd. (Sutton, UK), sodium hexane sulphonate, sodium phosphate tribasic, dodecahydrate (>98%), bovine serum albumin (BSA), diclofenac sodium (DLF) and streptomycin sulphate (STP) were all purchased from Sigma Aldrich (Gillingham, UK). Acetonitrile (HPLC grade), glycerol (GLY), trismethylamine, calcium chloride dihydrate, ethanol (laboratory grade), sodium hydroxide and orthophosphoric acid (analytical grade) were all purchased from Fisher Scientific (Leicestershire, UK). Potassium phosphate monobasic (99+% extra pure) was purchased from Acros Organic Ltd. (New Jersey, USA).

2.2. Preparation of films

Prior to preparation of the films, experiments were performed to produce 0.7–1.2% (w/w) aqueous gels for POL to determine suitable concentrations based on clear uniform solutions with no lumps of undissolved polymer and ease of pouring (Boateng et al., 2009). Optimised aqueous gels comprising only POL (1%, w/w) and blends of POL with CAR in a weight ratio of 75/25 (yielding 1% (w/w) of total polymer) were subsequently prepared by stirring on a magnetic stirrer at 70 °C to form a uniform gel. The gel was poured into Petri dishes (86 mm diameter) and dried in an oven at 40 °C for 18 h and the resulting films examined visually for morphological defects (*e.g.* cracks, tears, stickiness and patches) which can affect the handling, testing and application of the films as well as its aesthetic appearance. Films were plasticised with 0–50% GLY (*i.e.* 0–100 mg in the gel) to obtain optimised formulations with suitable mechanical properties. The drug loaded (DL) gels were prepared by the addition of an ethanolic solution of DLF to a polymeric gel (as described above) at 70 °C to obtain a final DLF concentration of 10% (w/w) This gel was cooled to 40 °C with constant stirring and an aqueous solution of STP was subsequently added to achieve a final STP concentration of 30% (w/w). The DL gel was dried in an oven at 40 °C for 18 h as above, to obtain the DL films. The dried films were carefully peeled off from the Petri dish, wrapped in Parafilm® and kept in desiccators over silica gel at room temperature (18 °C).

2.3. Tensile characterisation by texture analysis (TA)

The mechanical (tensile) properties of the films (0.05–0.09 mm thickness) were evaluated using a TA HD *plus* (Stable Micro System, UK) texture analyser equipped with 5 kg load cell and *Texture Exponent-32*® software program computers). The films ($n = 3$) free from any physical defects (*e.g.* cracks, tears, stickiness and patches) were cut into dumb-bell shapes and stretched between two tensile grips at 6 mm/s using a trigger force of 0.09 N until the films broke and the tensile strength, elastic modulus and elongation at break were determined.

2.4. Scanning electron microscopy (SEM)

Surface morphology of the films was analysed by a Hitachi SU 8030 (Hitachi High-Technologies, Germany) scanning electron microscope at low accelerating voltage (1 kV). Films were cut into small pieces and mounted on aluminium stubs (15 mm diameter) with 'Agar Scientific G3347N' double sided adhesive carbon tape. Images of the films were acquired at a working distance of 8.0 mm at a magnification of 2500×.

2.5. X-ray diffraction (XRD)

XRD was used to investigate the crystalline or amorphous nature of drugs (STP and DLF) and polymers at the surface of films and the effect of the plasticiser. The films were cut to fit the square tiles of the holder, mounted on the sample cell and scanned between 2θ of 2° to 45° with counting time of 0.1 s step size. The same was repeated for the pure drugs and polymers. X-ray patterns of the films and starting materials were obtained with DIFFRAC plus (Bruker Coventry, UK) having an XRD commander programme. A Goebbel mirror was used as monochromator which produced a focused monochromatic $CuK_{\alpha 1\&2}$ primary beam ($\lambda = 1.54184$ Å). The detector used for performing the experiment was Lynx Eye.

2.6. Differential scanning calorimetry (DSC)

Differential scanning calorimetry analysis was carried out on a DSC1 Mettler Toledo instrument (Leicester, UK). Films were cut into small pieces and 3–5 mg of sample placed into a 40 μl aluminium pan with lids and heated from −50 °C to 350 °C at the rate of 10 °C/min under constant purge of nitrogen (100 ml/min). The same was repeated for pure POL, CAR, STP and DLF.

2.7. Fourier transform infrared spectroscopy (FTIR)

A FTIR spectrophotometer was used in combination with (Thermo Nicolet, Thermoscientific, UK), ZnSe attenuated total reflectance (ATR) accessory to characterise the uniformity of the films. The FTIR was equipped with potassium bromide (KBr) beam splitter and MCT/A detector. The films were placed on an ATR crystal and maximum pressure was applied by using a pressure clamp accessory to allow for intimate contact of the films with the ATR crystal. Similarly, the pure starting materials (POL, CAR, STP and DLF) were analysed as controls. Spectra were recorded at 4 cm^{-1} resolution within a range of 650–4000 cm^{-1} using OMNIC®