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Preparation, optimisation and characterisation of novel wound healing film dressings loaded with streptomycin and diclofenac

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ABSTRACT

Streptomycin (STP) and diclofenac (DLF) loaded film dressings were prepared by blending Polyox® (POL) with four hydrophilic polymers [hydroxypropylmethylcellulose (HPMC), carrageenan (CAR), sodium alginate (SA) or chitosan (CS)] using glycerol (GLY) as plasticiser. The films were characterised by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy, texture analysis (tensile and swelling characteristics) and *in vitro* dissolution profiles using Franz diffusion cell. SEM showed homogeneous morphology for both blank (BLK) and drug loaded (DL) films. Films prepared by blending of POL with the other polymers showed a reduction in the crystallisation of POL in descending order of SA > CS > HPMC > CAR respectively. DSC and XRD showed no crystalline peaks of STP and DLF suggesting molecular dispersion of both drugs as well as possible drug interaction with negatively charged sulphate ions present in CAR. The DL films did not show any IR bands of both drugs, confirming the DSC and XRD results. POL-CAR-BLK films showed higher tensile strength (12.32 ± 1.40 MPa) than the POL-CAR-DL films (9.52 ± 1.12 MPa). DL films plasticised with 25% w/w GLY revealed soft and tough (tensile strength 1.02 ± 0.28 MPa, % elongation 1031.33 ± 16.23) formulations. The swelling capacities of POL-CAR-BLK and POL-CAR-DL films were (733.17 ± 25.78%) and (646.39 ± 40.39%), increasing to (1072.71 ± 80.30%) and (1051 ± 86.68%) for POL-CAR-BLK-25% GLY and POL-CAR-DL-25% GLY respectively. POL-CAR-DL films showed significantly ($n = 3$, $p < 0.0318$) lower cumulative release of STP and DLF (52.11 ± 1.34, 55.26 ± 2.25) compared to POL-CAR-DL-25% GLY films (60.07 ± 1.56, 63.39 ± 1.92) respectively.

1. Introduction

Recent advances in the use of wound dressings as drug delivery systems to improve wound healing have been extensively studied [1]. A wide range of wound dressings are frequently applied for a variety of wound types and to target the different stages of the wound healing process [2]. It has been suggested that wound healing involves a series of overlapping molecular events requiring extensive communication between cells and various physiological processes. These comprise haemostasis and inflammatory phase which begin immediately after wounding and continue for 3–4 days. The proliferative phase starts at about day 3 and persists for one to 3 weeks after injury and is characterised by fibroblast migration, deposition of extracellular matrix and formation of granulation tissue [2,3]. The remodelling and scar maturation phase starts at about week three and lasts for several weeks and includes synthesis and remodelling of extracellular matrix by concurrent development of granulation tissue which continues for prolonged periods [4,5].

Major limitations with wound management formulations such as creams and gels include their inability to maintain effective drug concentrations for a prolonged period at moist wound surfaces due to their short residence time. They are also associated with leakage and messiness causing inconvenience to patients which results in poor compliance [6]. Furthermore, dry traditional dressings such as gauze and cotton wool have limitations due to their inability to preserve a moist environment for effective wound healing [2]. On the other hand, more modern dressings such as hydrogels, hydrocolloids and films achieve effective wound healing by providing an optimum moist microenvironment for healing [3,7]. Desirable characteristics of dressings required for wound healing include: allowing gaseous exchange of O_2 and CO_2, maintaining a balanced moist environment (avoiding either maceration of healthy tissue or

Abbreviations: CAR, carrageenan; CS, chitosan; DLF, diclofenac; DL, drug loaded; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; GLY, glycerol; HPMC, hydroxypropylmethylcellulose; POL, Polyox®; SA, sodium alginate; SEM, scanning electron microscopy; STP, streptomycin; XRD, X-ray diffraction.

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wound desiccation) and allowing the evaporation and drainage of wound exudates. Others include the protection of the wound from physical damage and secondary infection, preventing wound contamination and peripheral channelling into the wound by bacteria, assisting in debridement, thermal insulation and ease of removal without causing any trauma to the wound [2,7,9].

Films prepared from hydrophilic bioadhesive polymers are also used for delivering drugs to moist surfaces such as wounds and the buccal cavity due to their biodegradable nature [8,9]. Dobaria et al. found that bioadhesive polymers have the capacity to adhere to mucosal epithelial surfaces [6] and thereby prolong contact time and subsequently prolonged drug release. Combining bio (natural) polymers with synthetic polymers (which on their own do not always meet all the complex demands within a biological system) is of particular significance due to the biocompatibility imparted by the natural polymers [10]. On the other hand, though biopolymers exhibit biocompatibility characteristics, they often possess poor mechanical properties which can make application onto a mucosal surface such as a wound very challenging [10]. Polyox® (POL) (polyethylene oxide) is a synthetic uncharged polymer with molecular formula $(-CH_2CH_2O-)_n$. It is semi-crystalline and bioadhesive due to its water solubility, high viscosity, ability to form hydrogen bonds and compatibility with other bioactive substances [8]. It has been shown that POL exhibits increased mucoadhesive capacity and can be used as a drug carrier with improved performance [11].

According to Zivanovic et al. [8], films prepared from synthetic polymers such as polyethylene oxide have relatively poor physical characteristics such as stickiness and high water solubility which limit their application. Generally, formation of specific intermolecular interactions through weak hydrogen bonding between two or more polymers is responsible for the observed behaviour of formulations prepared from aqueous gels comprising blends of polymers [10]. Studies by Kondo and Sawatari established that primary hydroxyl groups on cellulose and methylcelluloses can form hydrogen bonds with the ether oxygen of polyethylene oxide (POL) [12]. Analogous studies based on this principle were carried out using polyethylene oxide with sodium alginate and starch respectively. Similarly, hydroxyl groups on carrageenan can form hydrogen bond with the ether oxygen in POL [10,13]. The advantage of such an approach lies in a reduction in the limitations of each individual polymer whilst maximising the optimum properties of each polymer within the combined entity.

Combined therapy using drugs with different therapeutic and pharmacological actions is an effective way to achieve optimum and rapid wound healing with minimum inflammatory responses [14]. For example, antibiotic drugs such as streptomycin (STP) can prevent as well as treat wound infections whilst anti-inflammatory drugs such as diclofenac (DLF) can target the inflammatory phase of wound healing and relieve the swelling and pain associated with injury. DLF is also reported to possess moderate antibacterial activity both *in vitro* and *in vivo* in part from its ability to inhibit DNA synthesis of bacteria [15]. DLF was found to possess antibacterial activity against both drug sensitive and drug resistant clinical isolates of *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Mycobacterium* spp, in addition to its potent anti-inflammatory activity [15,16]. It also possesses anti-plasmid activity and acts as a 'helper compound' in synergistic combination with STP against *E. coli* [15].

In this study, we report on film dressings formulated by blending Polyox® (POL) with four different hydrophilic polymers, namely carrageenan (CAR), sodium alginate (SA), chitosan (CS), hydroxypropylmethylcellulose (HPMC). All polymers were chosen due to their well known bioadhesive and film forming properties. The films were characterised using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Further, films plasticised with glycerol (GLY) (0–100% w/w) were characterised by measuring their tensile properties on a texture analyser. Two model drugs (STP and DLF) which target two different stages of wound healing were incorporated into the optimised plasticised films. Swelling and *in vitro* drug release studies by Franz diffusion cell were conducted at 37 °C using phosphate buffer.

2. Experimental

2.1. Materials

Polyox® WSR 301 LEO NF (≈4000 KDa) was obtained from Colorcon Ltd; (Dartford, UK). kappa carrageenan (CAR) (Gelcarin GP 812 NF) was obtained from IMCD Ltd (Sutton, UK). Chitosan (CS) (medium molecular weight 9413) 75–85% deacetylated, sodium hexane sulphonate, sodium phosphate tribasic dodecahydrate (>98%), glycerol (GLY) (approximately 98%), hydroxypropylmethylcellulose (HPMC), diclofenac sodium (DLF) and streptomycin sulphate (STP) were all purchased from Sigma Aldrich (Gillingham, UK). Sodium alginate (SA), acetonitrile (HPLC grade), 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 obtained from Acros Organic Ltd (New Jersey, USA).

2.2. Preparation of films

Blank (BLK) polymeric films were prepared by solvent casting method. Aqueous gels comprising only POL (1% w/w) and blends of POL separately with CAR, CS, SA and HPMC in a weight ratio 75/25 (1% w/w of total polymer) were prepared by stirring on a magnetic stirrer with heating at 70 °C. For CS, which is insoluble in water, the pH was adjusted using 1% v/v acetic acid solution to ensure complete dissolution. The resulting gels were poured into Petri dishes (86 mm diameter) and kept in an oven at 40 °C for 18 h to dry and the films obtained were examined visually for morphological defects. Selected formulations (gels) were then loaded with 0–100% w/w GLY as plasticiser. A 4 ml ethanolic solution of DLF containing 100 mg of the drug (2.5% w/v solution) was added to the gel to achieve a final DLF concentration of 10% w/w in the polymeric gel and cooled to 40 °C with constant stirring. Similarly, 4 ml aqueous solution containing 300 mg of STP (7.5% w/v solution) was subsequently added to achieve a final STP concentration of 30% w/w in the drug loaded (DL) gels. The DL gel was dried in an oven at 40 °C for 18 h as above to obtain the DL films.

The composition of the dried film (mg) for the polymers, drugs and plasticiser present in the films are summarised in Table 1. The dried films were carefully peeled off from the dish, wrapped in parafilm and kept in desiccators over calcium chloride at room temperature and subsequently analysed for their physico-chemical, mechanical (tensile), swelling and *in vitro* drug release properties. The thickness of all the films were measured at five different locations (centre and four areas around the edges) using a micrometer screw gauge, and mean thickness was calculated.

2.3. Scanning electron microscopy (SEM)

Surface morphology of the films was analysed by a Cambridge S-360 (gSEMI, California, USA) scanning electron microscope at low accelerating voltage. The films were mounted onto 'Agar Scientific G301' aluminium pin-type stubs (12 mm diameter) using 'Agar Scientific G3347N' double sided adhesive carbon tape. These films were then sputter coated with gold (Edwards 188 Sputter Coater S150B) before placing in the chamber of the microscope and images acquired using an i-scan2000 software.