Multifunctional Medicated Lyophilised Wafer Dressing for Effective Chronic Wound Healing

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ABSTRACT: Wafers combining weight ratios of Polyox with carrageenan (75/25) or sodium alginate (50/50) containing streptomycin and diclofenac were prepared to improve chronic wound healing. Gels were freeze-dried using a lyophilisation cycle incorporating an annealing step. Wafers were characterised for morphology, mechanical and *in vitro* functional (swelling, adhesion, drug release in the presence of simulated wound fluid) characteristics. Both blank (BLK) and drug-loaded (DL) wafers were soft, flexible, elegant in appearance and non-brittle in nature. Annealing helped to improve porous nature of wafers but was affected by the addition of drugs. Mechanical characterisation demonstrated that the wafers were strong enough to withstand normal stresses but also flexible to prevent damage to newly formed skin tissue. Differences in swelling, adhesion and drug release characteristics could be attributed to differences in pore size and sodium sulphate formed because of the salt forms of the two drugs. BLK wafers showed relatively higher swelling and adhesion than DL wafers with the latter showing controlled release of streptomycin and diclofenac. The optimised dressing has the potential to reduce bacterial infection and can also help to reduce swelling and pain associated with injury due to the anti-inflammatory action of diclofenac and help to achieve more rapid wound healing. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1720–1733, 2014

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INTRODUCTION

According to the Wound Healing Society, a wound is the consequence of disruption of normal anatomic structure and function. It usually describes the rupture or defect in skin or body tissue due to physical or thermal damage or a consequence of underlying physiological and medical conditions.[1] The wound healing process is a complex phenomenon and involves different phases such as haemostasis, inflammation, proliferation, remodelling and scar maturation which are discussed elsewhere.[1–3] Based on the nature of the repair process, wounds are classified as acute and chronic. Compared with acute wounds, chronic wounds represent a medical challenge because of various complicating factors including diabetes and malignancies, chronic systemic inflammation, persistent infection, destruction of neighbouring tissues, poor primary treatment and other patient-related factors such as poor nutrition.[4]

The management of chronic wounds places an enormous drain on healthcare resources; with some studies estimating the cost of wound care management to the UK National Health Service (NHS) to be about £1 billion a year. In the UK, around 24,000 admissions a year involve patients with diabetic foot ulceration alone, thereby costing the NHS some £17 million.[5] Winter's theory of wound healing introduced a new approach for achieving rapid wound healing by maintaining a moist environment around the wound.[6] This principle of moist wound healing formed the basis of increased demand for developing a new range of modern wound dressings that can absorb excess of exudate and allow the maintenance of adequate moisture at wound surfaces. Further, different types of wounds (e.g. acute, chronic, exuding and dry wound) also affect the choice of dressing and in fact, no single dressing fulfils all the requirements (ideal characteristics) suitable for the management of all wounds.[1]

Wound exudate from acute wounds contains many endogenous substances which typically reflect the overall wound healing process. These include epithelial and fibroblast cells which have been shown to increase the rate and quality of wound healing.[7] On the other hand, most chronic wound exudates are associated with bacteria, dead white cells in combination with high levels of inflammatory mediators and protein-digesting enzymes which can be unfavourable for the wound healing process.[8] In modern wound care practice, iodine, silver and broad spectrum germicidal agents such as neomycin, bacitracin, polymyxin, streptomycin sulphate (STP), gentamycin and/or combinations are used to control and treat bacterial infection in chronic wounds. Local delivery of these antibiotics in the form of dressings is more convenient over their systemic counterparts as they deliver a higher concentration of

Abbreviations used: ATR, attenuated total reflectance; BLK, blank; BSA, bovine serum albumin; CAR, carrageenan; DLF, diclofenac sodium; DL, drug loaded; DSC, differential scanning calorimetry; POL, Polyox™; SA, sodium alginate; SEM, scanning electron microscopy; STP, streptomycin sulphate; SWF, simulated wound fluid; XRD, X ray diffraction; WOA, work of adhesion.

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medication directly to the desired area and are less frequently implicated in causing bacterial resistance.[9]

Polysaccharides, being naturally occurring biomolecules, are an obvious choice for application as potential wound management aids.[10] It has been previously demonstrated that the use of synthetic and natural polymers helps to improve the properties which makes them suitable for application in the biomedical field.[11] Pawar et al.[12] prepared films from blends of synthetic and natural polymers for potential improvement in chronic wound healing. However, highly exuding chronic wounds such as diabetic foot and venous ulcers limit the application of film dressings because of the high amount of exudate produced. Film dressings being poor at absorbing large volumes of exudate, allow the fluid to collect beneath the dressing, causing maceration at the wound site and therefore require frequent dressing changes which adversely affects patient compliance.

Lyophilised wafers are produced by freeze-drying polymer solutions and gels to yield solid porous structures that can easily be applied to exuding wound surfaces.[13] It is anticipated that a lyophilised polymer matrix would preserve the size, shape and form of contained compounds unlike a conventional gel suspension, where crystal ripening, agglomeration and polymorphic changes may occur.[14] Their physical architecture resembles those of foam dressings which are made of porous polyurethane. Drug stability is better in a lyophilised dosage form compared with a semi-solid hydrogel-based formulation.[15] Lyophilised wafers provide a potential means of delivering pharmacological agents to wound surfaces to aid healing.[16] They have the ability to incorporate soluble and insoluble antimicrobial compounds greater than their minimum bactericidal concentration for antibacterial activity against pathogenic bacteria.[17] Wafers have the capacity to absorb large amounts of exudate because of their porous nature whilst maintaining a moist environment without damaging newly formed tissue. Wafers also offer high drug-loading capacity compared with the solvent cast films.[18]

This study involves preparation and functional characterisation of lyophilised wafers of Polyox (POL) in combination with carrageenan (CAR) or sodium alginate (SA) loaded with streptomycin (antibacterial) and diclofenac (anti-inflammatory) to target infection and the inflammatory phase of wound healing. The prepared wafers were characterised by scanning electron microscopy (SEM), X-ray diffraction (XRD), FTIR and mechanical properties using a texture analyser. The optimised wafers were further evaluated for functional bio-analytical properties such as swelling, adhesion and drug release properties.

EXPERIMENTAL

Materials

Polyethylene oxide (Polyox™ WSR 301 ≈ 4000 kDa) was obtained as a gift from Colorcon Ltd. (Dartford, UK); κ-CAR (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 STP were all purchased from Sigma–Aldrich (Gillingham, UK). SA, acetonitrile (HPLC grade), glycerol, tris(hydroxy)aminomethane, calcium chloride dihydrate, ethanol (laboratory grade) and orthophosphoric acid (analytical grade) were all purchased from Fisher Scientific (Leicestershire, UK).

Table 1. Composition of Polymers and Drugs in Gels Used for Freeze-Dried Wafers

Starting Material	POL–CAR–BLK	POL–CAR-DL Weight (g)	POL–SA–BLK	POL–SA-DL
POL	0.75	0.75	0.50	0.50
CAR	0.25	0.25	–	–
SA	–	–	0.50	0.50
STP	–	0.30	–	0.25
DLF	–	0.25	–	0.10
Total weight	1.00	1.55	1.00	1.35

The final polymer concentration was 1% (w/w).

Preparation of POL–CAR and POL–SA Gels

Blank (BLK) polymeric gels (1% w/w) of POL and CAR and POL and SA were prepared according to previously reported methods.[12,19] In brief, blends of POL with CAR and POL with SA (weight ratio of 75/25 and 50/50) respectively yielding 1% (w/w) of total polymer weight, were prepared by stirring on a magnetic stirrer at 70°C to form a uniform gel. The drug-loaded (DL) gels were prepared by the addition of an ethanolic solution of DLF to the polymeric gel (as described above) at 70°C to obtain a final DLF concentration of 10% and 25% (w/w) respectively for POL–SA and POL–CAR gels. The gel was subsequently cooled to 40°C with constant stirring and an aqueous solution of STP was subsequently added to achieve a final STP concentration of 25% and 30% (w/w) respectively for POL–SA and POL–CAR gels. The amounts of the polymers and drugs used for the preparation of gels are summarised in Table 1.

Freeze-Drying Cycle Development

Prior to lyophilisation, preliminary DSC studies on the BLK (POL–CAR and POL–SA) gels were carried out. A differential scanning calorimeter DSC-1 (Mettler Toledo Ltd., Leicester, UK), calibrated with indium (at 10°C/min) was used to analyse the thermal events in the gels to determine a more suitable lyophilisation cycle. The BLK gels were cooled in 40 μL aluminium pans (ME-00026763; Mettler Toledo Ltd.) from 25°C to −60°C at a rate of 10°C/min. They were then re-heated back to 25°C at 20°C/min and the cycle repeated three times. Based on thermal events observed during the heating cycles, an annealing temperature of −25°C was chosen. The samples were then cooled to −60°C, warmed to −25°C, held at that temperature for 10 min, cooled back to −60°C and then warmed through to 25°C at 20°C/min.

Wafer Preparation

The freeze-dried wafers were prepared by freeze-drying (10 g) of each homogeneous gel in six-well moulds (diameter 35 mm) (Corning® CellBIND®; Sigma–Aldrich) in a Virtis Advantage XL 70 freeze dryer (Biopharma Process Systems, Winchester, UK) using an automated novel lyophilisation cycle (Fig. 1). This involved initially cooling and freezing (including annealing step) for samples from room temperature to −5°C and then −50°C over a period of 10 h (at 200 mTorr). An annealing step at −25°C for 2 h was applied and its effect on the DL formulation investigated. The frozen samples were then heated in a series of thermal ramps to −25°C under vacuum (20–50 mTorr) over a 24 h period. Secondary drying of the wafers was carried