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Comparison of *in vitro* antibacterial activity of streptomycin-diclofenac loaded composite biomaterial dressings with commercial silver based antimicrobial wound dressings

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ABSTRACT

Infected chronic wounds heal slowly, exhibiting prolonged inflammation, biofilm formation, bacterial resistance, high exudate and ineffectiveness of systemic antimicrobials. Composite dressings (films and wafers) comprising polyox/carrageenan (POL-CAR) and polyox/sodium alginate (POL-SA), loaded with diclofenac (DLF) and streptomycin (STP) were formulated and tested for antibacterial activity against 2×10^5 CFU/mL of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* representing infected chronic wounds and compared with marketed silver dressings. Minimum inhibitory concentration (MIC) showed higher values for DLF than STP due to non-conventional antibacterial activity of DLF. The DLF and STP loaded dressings were highly effective against *E. coli*, *P. aeruginosa* and *S. aureus*. POL-SA dressings were more effective against the three types of bacteria compared to POL-CAR formulations, while the DLF and STP loaded dressings showed greater antibacterial activity than the silver-based dressings. The films, showed greater antibacterial efficacy than both wafers and silver dressings. STP and DLF can act synergistically not only to kill the bacteria but also prevent their resistance and biofilm formation compared to silver dressings, while reducing chronic inflammation associated with infection.

1. Introduction

A wound is an interruption in the defensive role of the skin in protecting against harmful environmental agents [1]. Injury evokes wound healing comprising distinct phases (haemostasis, inflammation, proliferation, migration and maturation) involving biochemical, and molecular events that work sequentially towards tissue regeneration [2]. However, wounds can get contaminated by microorganisms, especially during the proliferation stage leading to infection. Persistent infection impairs wound healing causing repeating inflammatory cycle, resulting in chronic wounds [3,4]. Prevention and control of infection have been identified as essential aspects of wound management [5]. Effective management requires reducing exogenous microbial contamination, debridement, using appropriate dressing(s) and administration of topical and systemic broad-spectrum antimicrobial agents [6]. Topical agents such as povidone iodine and chlorhexidine acid are commonly employed, though their use is currently restricted to wound cleansing and skin swabs before surgical incisions [1]. However, antibiotics have high specificity against infection and ultimately improve wound healing at low concentrations [1,7]. Various commercial dressings have been developed that release silver to prevent wound infections both *in vitro* [8] and *in vivo* [9]. The emergence of microbial resistance has resulted in the need for more effective treatments for wound infections [1]. Further, systemic antibiotic treatment is difficult in chronic wounds such as diabetic foot ulcers due to poor blood circulation at the extremities of diabetics [6].

Chronic wound infection also causes pain, excessive exudation and patient discomfort and is a major source of cross-infection particularly antibiotic-resistant species. Burns for example provide a protein-rich environment, favourable for microbial colonization [10]. Most infected wounds involve *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococci* and *Escherichia coli*. *S. aureus* is considered a challenging microorganism in wound infections [6] due to its ability to develop resistance against first line antibiotics.

Streptomycin (STP) has been used to treat wound infections [11] and for reducing infection before skin grafting [12]. It's reported that diclofenac (DLF) has antibacterial activity and acts synergistically with STP against Mycobacterium tuberculosis after systemic administration [13]. Systemic STP in combination with DLF demonstrated synergistic activity against 45 different strains of mycobacteria [14,15].

This paper reports on the evaluation of antibacterial activity of STP and DLF loaded film and wafer dressings against *S. aureus*, *E. coli* and *P. aeruginosa*. Minimum inhibitory concentration (MIC) of STP and DLF in the dressings and *in vitro* antibacterial efficacy (zone of inhibition) against the three microorganisms were evaluated using disk diffusion

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assay and compared with three commercial silver containing dressings. To the best of our knowledge, this is the first study comparing the antibacterial performance of STP-DLF loaded medicated POL-CAR and POL-SA dressings with commercial silver loaded antimicrobial dressings for their antibacterial performance.

2. Methods

2.1. Materials

(Polyox™ WSR 301 ≈ 4000 kDa) was a gift from Colorcon Ltd. (Dartford, UK), κ-carrageenan (Gelcarin GP 812) was from IMCD Ltd. (Sutton, UK), Aquacel® Ag (ConvaTech, Ltd.), Melgisorb® Ag (Mölnlycke Health Care, Ltd.) were gifted by the manufacturers and Allevyn® Ag (Smith and Nephew, Ltd) obtained from a local pharmacy. Nutrient agar and nutrient broth were purchased from Oxoid, UK. Diclofenac sodium, streptomycin sulphate, glycerol, phosphate buffered saline (PBS) tablets, were purchased from Sigma-Aldrich, (Gillingham, UK). Sodium alginate was purchased from Fisher Scientific (Loughborough, UK). National Collection of Type Culture (NCTC) strains of *S. aureus* (A 29213), *E. coli* (DTCC 25922) and *P. aeruginosa* (A 10145), were used for microbiological assays.

2.2. Preparation of composite polymer based dressings

Composite films and wafers (Table 1) were prepared as previously reported [16,17]. 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 (POL-CAR-BLK and POL-SA-BLK). The composition of the polymers and drugs used for the preparation of gels are summarised in Tables 2 and 3. DL gels of POL-SA and POL-CAR were prepared by adding 4 mL ethanolic solution of DLF containing 100 mg and 250 mg respectively of the drug to POL-SA gel to achieve 10% w/w and to achieve 25% w/w of DLF for POL-CAR in the polymeric gel. These gels were subsequently cooled to 40 °C with constant stirring. Similarly, a 4 mL aqueous solution containing 250 mg and 300 mg of STP was subsequently added to achieve a final STP concentration of 25% w/w (POL-SA) and 30% w/w (POL-CAR) respectively in the DL gels.

To obtain films, the solutions (25 g) were poured into Petri dishes (diameter 90 mm) and dried in an oven at 40 °C for 18 h, while unplasticised polymeric solutions (10 g) were freeze-dried to obtain wafers. To obtain the wafers, 10 g of each homogeneous gel was transferred into 6 well moulds (diameter 35 mm) (Thermo-Fisher Scientific Nunc, Leicestershire UK), placed in a Virtis Advantage XL 70 freeze dryer (Biopharma Process Systems, Winchester, UK) and lyophilised using the automated lyophilisation cycle. This involved initially cooling 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 based on the preliminary DSC studies and its effect on the different formulations was investigated. The frozen samples were then heated in a series of thermal steps to −25 °C under vacuum (20–50 mTorr) over a 24 h period. Secondary drying of the wafers was carried out at 20 °C (10 mTorr) for 7 h.

Table 1
Formulations used to evaluate antimicrobial efficacy against *S. aureus*, *P. aeruginosa* and *E. coli*.

Formulation	Code
POL-CAR-BLK	A
POL-CAR-DL	B
POL-CAR-DL-20%GLY	C
POL-SA-BLK	D
POL-SA-DL	E
POL-SA-DL-9%GLY	F
POL-CAR-BLK-An	G
POL-CAR-DL-An	H
POL-SA-BLK-An	I
POL-SA-DL-An	J
Aquacel® Ag	K
Melgisorb® Ag	L
Allevyn® Ag	M
STP	N
DLF	O

Table 2
Quantities of the polymers, drugs and GLY (varying amounts based on total solid weight) within composite polymer gels used for formulation of POL-CAR and POL-SA (BLK and DL) films.

Formulation	POL (g)	CAR (g)	SA (g)	GLY (g)	DLF (g)	STP (g)	Total weight (g)	% GLY content
POL-CAR-BLK	0.75	0.25	–	0.00	–	–	1.00	0.00
POL-CAR-BLK	0.75	0.25	–	0.10	–	–	1.10	9.09
POL-CAR-BLK	0.75	0.25	–	0.25	–	–	1.25	20.00
POL-CAR-BLK	0.75	0.25	–	0.50	–	–	1.50	33.33
POL-CAR-BLK	0.75	0.25	–	0.75	–	–	1.75	42.86
POL-CAR-BLK	0.75	0.25	–	1.00	–	–	2.00	50.00
POL-CAR-DL	0.75	0.25	–	0.00	0.10	0.30	1.40	0.00
POL-CAR-DL	0.75	0.25	–	0.10	0.10	0.30	1.50	6.67
POL-CAR-DL	0.75	0.25	–	0.25	0.10	0.30	1.65	15.15
POL-CAR-DL	0.75	0.25	–	0.50	0.10	0.30	1.90	26.32
POL-CAR-DL	0.75	0.25	–	0.75	0.10	0.30	2.15	34.88
POL-CAR-DL	0.75	0.25	–	1.00	0.10	0.30	2.40	41.67
POL-SA-BLK	0.50	–	0.50	0.00	–	–	1.00	0.00
POL-SA-BLK	0.50	–	0.50	0.10	–	–	1.10	9.09
POL-SA-BLK	0.50	–	0.50	0.25	–	–	1.25	20.00
POL-SA-BLK	0.50	–	0.50	0.50	–	–	1.50	33.33
POL-SA-DL	0.50	–	0.50	0.00	0.05	0.15	1.20	0.00
POL-SA-DL	0.50	–	0.50	0.10	0.05	0.15	1.30	7.69
POL-SA-DL	0.50	–	0.50	0.25	0.05	0.15	1.45	17.24
POL-SA-DL	0.50	–	0.50	0.50	0.05	0.15	1.70	34.48

2.3. Bacterial sample preparation

Fresh broth cultures were prepared as reported by Labovitiadi et al., [18] by transferring a single bead unit into 10 mL of nutrient broth and incubating for 24 h. A loop full of bacterial culture was streaked onto nutrient agar plate and incubated at 37 °C for 24 h to yield separate colonies. Overnight bacterial cultures were centrifuged at 4000 rpm for 10 min in an Accuspin 1 centrifuge (Fisher Scientific, UK), supernatant discarded and pellets suspended in 20 mL of simulated wound fluid (SWF) [16]. This process was repeated twice and final pellets resuspended in 5 mL SWF, followed by two fold dilutions in SWF. Bacterial density was determined by measuring the dilute suspension at 500 nm to yield the required density of 2×10^5 CFU/mL [18].

2.4. Minimum inhibitory concentration (MIC) of STP and DLF

The MIC for STP and DLF was evaluated as previously reported [19]. Briefly, three different stock solutions for each drug were prepared (Table 4) and STP required to obtain 10,000 mg/L was calculated using Eq. (1). Antimicrobial susceptibilities of *S. aureus*, *E. coli* and *P.*

Table 3
Composition of polymers and drugs (varying quantity) present in composite polymer gels used to produce composite freeze dried POL-CAR and POL-SA (BLK and DL) wafers.

Pure material	POL-CAR-BLK (g)	POL-CAR-DL (g)	POL-SA-BLK (g)	POL-SA-DL (g)
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 (g)	1.00	1.55	1.00	1.35