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Research paper

A biocompatible sodium alginate/povidone iodine film enhances wound healing

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ABSTRACT

In the last few years, there has been an increasing tendency to use natural polymers for the fabrication of dressings for wound and burn management. Among them, alginate, a polysaccharide extracted primarily from marine algae, exhibits attractive properties being non-toxic, hydrophilic and biodegradable. The aim of this study was to characterize the *in vitro* biocompatibility and the efficacy of a composite polymeric material based on sodium alginate (NaAlg) and povidone iodine (PVPI) complex in a mouse model of wound healing. The developed material combines the excellent wound healing properties of alginates with the bactericidal and fungicidal properties of PVPI, providing a controlled antiseptic release. We demonstrated that the NaAlg/PVPI films are able to reduce the inflammatory response both in human foreskin fibroblasts after lipopolysaccharide (LPS) stimulus and in rodents after wound induction. Furthermore, the NaAlg/PVPI film-treated animals showed a significantly higher wound closure compared to untreated animals at each time point considered. Interestingly, the complete wound closure was achieved within 12 days only in the film-treated group, indicating that the full-thickness wounds healed more rapidly in these animals. The results demonstrate that the NaAlg/PVPI films are biocompatible and possess healing properties that accelerate the wound closure.

1. Introduction

Wound healing is a complex and highly regulated physiological process that involves various cell types i.e. immune cells, endothelial cells, keratinocytes, and fibroblasts) and pathways, activated and coordinated in order to restore tissue integrity and homeostasis [14]. This dynamic process can be divided into four, partly overlapping, phases: hemostasis, inflammation, proliferation and maturation [4]. After injury, bleeding occurs and this vascular response helps to cleanse the wound of surface contaminants (e.g. bacteria and/or antigens). Hemostasis results from the activation of platelets, which starts the coagulation cascade and platelet aggregation to form the hemostatic blood clot that acts as a temporarily protective barrier for the injured skin [31]. The inflammatory phase occurs almost simultaneously with hemostasis, within 24 h and lasts for four – six days. This phase is mediated by neutrophils and monocytes that differentiate into macrophages) [5]. Neutrophils are involved in infection control, cleaning of the wound from foreign particles e.g. debris, bacteria) together with macrophages. Moreover, both these cell types release various cytokines, chemokines, and enzymes that activate fibroblasts and myofibroblasts in the proliferation phase of wound healing [8]. These molecules, together with a variety of nutrients, are contained in the wound exudate, which provides the moist environment important for effective healing. In the proliferation phase, epithelialization occurs and newly formed granulation tissue, consisting of endothelial cells, macrophages, and fibroblasts begin to cover and fill the wound area by producing new extracellular matrix (ECM) required for sustaining cells and blood vessels, which provide nutrients needed to restore tissue integrity. Finally, during the maturation (also called “remodeling phase”), collagen forms tight cross-links with other collagen and protein molecules, increasing the tensile strength of the scar [31].

Delayed or impaired wound healing has a significant socio-economic burden for patients and health-care systems worldwide [30]. Multiple factors e.g. desiccation, oxygenation, transpiration, and infections) and pathological conditions e.g. diabetes, obesity, and cancer) can lead to impaired wound healing, increasing the incidence of chronic wounds [13]. In particular, some common features shared by these conditions include a prolonged or excessive inflammation [10],

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persistent infections [9], formation of drug-resistant microbial biofilms [36], and the inability of dermal and/or epidermal cells to respond to reparative stimuli. Moreover, the excessive exudate production associated to abnormal inflammation can cause skin sensitivities and tissue maceration and infection [7]. Elimination or reduction of prolonged inflammation revitalizes tissue healing, reduces exudate and is usually associated with bioburden reduction.

For these reasons, the design of advanced therapeutic dressing, that play an active role in promoting healing of both acute and chronic wounds, represents a promising approach to fulfil the requirements needed for the next generation wound care. Bioactive natural polymers are commonly used as wound dressings because of their biocompatibility, biodegradability, mechanical properties, bioresorption ability, and relatively low-cost [22]. Among them, NaAlg, a polysaccharide derived from brown seaweed, is the most widely investigated and used in biomedical applications. Alginate is highly hydrophilic and able to absorb wound exudate, maintaining a moist microenvironment. Moreover, the anti-inflammatory activity of NaAlg electrospun nanofibers was recently reported [15]. Alginate dressings are also useful as delivery platform in order to provide a controlled release of therapeutic substances (e.g. pain-relieving, antibacterial and anti-inflammatory agents) to exuding wounds [6,21,33].

PVPI is one of the topical antiseptic agents most commonly used in wound care [1]. PVPI is a water-soluble complex of iodine and polyvinylpyrrolidone, a broad-spectrum antiseptic agent effective against a wide variety of bacteria, viruses, fungi, protozoa and yeasts. Despite its diffusion as topical antiseptic agent, PVPI solution showed toxic effects on human skin fibroblast, totally inhibiting cell growth at 0.1% and 1.0% concentrations [2], suggesting a potential damaging effect towards cells involved in wound repair. In order to overcome this harmful effect, we have incorporated PVPI into alginate matrix to produce NaAlg/PVPI composite films, allowing its time controlled release at the wound surface. Moreover, we have demonstrated that these films have antimicrobial and antifungal activities against *Escherichia coli* and *Candida albicans*, respectively [18].

In the choice of a wound dressing material, its biocompatibility represents one of the most important features, in particular for bioactive polymers that can interact with the wound environment and influence cell functions [26]. To this purpose, we evaluated NaAlg/PVPI films biocompatibility on human foreskin fibroblast (HFF-1) and assessed their potential anti-inflammatory effect in a Lipopolysaccharide (LPS)-stimulated inflammatory model, previously developed with the same cell line [15]. Dexamethasone, a synthetic glucocorticoid compound with potent anti-inflammatory activities, was used as positive control for interleukins reduction after LPS-stimulation [23]. We then went on to study the effect of NaAlg/PVPI films *in vivo*, applying them to a murine full-excisional skin wound model and exploring their efficiency in wound healing, measured as time and percentage of decrease of open wound area and as migration of epithelial tongue in the wound bed. We also evaluated the anti-inflammatory effect and hydroxyproline levels as an indicator of collagen content in the extracts of healing skin tissues. *In vitro* and *in vivo* studies were conducted in parallel on a commercial Povidone Iodine Non-Adherent Dressing product, used as standard of care for wound management.

2. Materials and methods

2.1. Materials and cell culture

Sodium alginate, PVPI (Poly(vinylpyrrolidone)–Iodine complex), lipopolysaccharides (LPS) from *Escherichia coli* (serotype 026:B6), dexamethasone (DXM) and Trypan Blue 0.4% solution (T8154) were purchased from Sigma Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine and penicillin–streptomycin were from Euroclone (Milan, Italy). Human foreskin fibroblasts (HFF-1) were from ATCC®. A commercial Povidone Iodine Non-Adherent Dressing product (named as Product A throughout the text) was used as standard of care for wound management. This dressing consists in a knitted viscose fabric impregnated with a polyethylene glycol (PEG) base containing 10% Povidone Iodine equivalent to 1% available iodine, which is released in the presence of wound exudate.

RNA extraction kit (PureLink RNA Mini Kit) and Super-Scripts® VILO™ cDNA Synthesis Kit were purchased from Ambion by Life Technologies (USA). Gene-specific primers using fluorogenic probes (TaqMan) and TaqMan® Universal PCR Master Mix, No AmpErase® UNG were from Applied Biosystems (USA).

2.2. Preparation of the NaAlg/PVPI films

NaAlg/PVPI films were prepared as previously described [18]. Briefly, 3 g of NaAlg were dissolved in 100 mL of distilled water under constant stirring for 1 h at 100°C. Then, 0.3 g of PVPI were added to NaAlg solution and dissolved under constant stirring for 1 h at room temperature, to obtain a final concentration of 10% by wt. PVPI. Finally, a volume of glycerol (corresponding to 5% of the starting NaAlg weight) was added to the NaAlg/PVPI solution and dissolved under constant stirring for 1 h at room temperature. Each film was cast with 10 mL of solution and dried for 24 h under a chemical hood.

2.3. Film characterization and extraction for in vitro studies

According to ISO10993-12 (sample preparation), an extraction ratio of 6 cm^2/mL (surface area/volume) should be used for preparation of test samples. To standardize the treatments, NaAlg/PVPI films (55 cm^2/film) were weighted and their thickness was measured using a digital micrometer. Five measurements were performed on different parts of the films. Average values with the corresponding standard deviation (S.D.) were calculated. Based on the average values obtained, 318.7 mg of films, corresponding to a surface area of 55 cm^2, were extracted in 9.2 mL of cell culture medium (containing 3.5 mg PVPI/mL) to perform both cytotoxicity and anti-inflammatory experiments. Extraction medium from NaAlg/PVPI films was prepared adapting the procedure described in ISO10993-5 standard test. Briefly, films were sterilized under a germicidal UV lamp (wavelength 253.7 nm) for 1 h (30 min/side), then samples were cut into small pieces to enhance submersion in the extraction medium. Extraction was performed in cell culture medium for 24 h at 37 °C and was used immediately after preparation.

Product A was cut into squares according to the following extraction ratios: 0.7 – 1.0–1.5 cm^2/mL, corresponding to 2.7 – 3.9–5.8 mg/mL PVPI, respectively. Extraction was performed in cell culture medium for 24 h at room temperature.

2.4. In vitro biocompatibility assay

HFF-1 cells were grown in DMEM supplemented with 10% FBS, 2 mmol L^{-1} L-glutamine, 100 IU per mL penicillin and 0.1 mg mL^{-1} streptomycin, in a humidified incubator at 37 °C with 5% CO_2. For biocompatibility experiments, HFF-1 cells were seeded in 60 mm cell culture dishes at a density of 3.5×10^5. After 24 h of culture, medium was replaced with the extraction one (control samples were treated with medium processed as the extractions) and cells were incubated for additional 24 h. Cell viability was assessed using the trypan blue exclusion assay. Based on dye uptake, viable (clear) and nonviable (blue) cells were counted using a hemocytometer and a light microscope. Cell viability was expressed as a percentage relative to control cells.

2.5. In vitro evaluation of anti-inflammatory properties

HFF-1 cells were seeded in 60 mm cell culture dishes at a density of 4×10^5 and cultured for 24 h. Then, cell culture medium was removed