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Structure–property effects of novel bioresorbable hybrid structures with controlled release of analgesic drugs for wound healing applications

Maoz Shemesh, Meital Zilberman *

Department of Biomedical Engineering, Faculty of Engineering, Tel-Aviv University, Tel-Aviv 69978, Israel

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ABSTRACT

Over the last decades, wound dressings have developed from the traditional gauze dressing to tissue-engineered scaffolds. A wound dressing should ideally maintain a moist environment at the wound surface, allow gas exchange, act as a barrier to micro-organisms and remove excess exudates. In order to provide these characteristics, we developed and studied bioresorbable hybrid structures which combine a synthetic porous drug-loaded top layer with a spongy collagen sublayer. The top layer, prepared using the freeze-drying of inverted emulsions technique, was loaded with the analgesic drugs ibuprofen or bupivacaine, for controlled release to the wound site. Our investigation focused on the effects of the emulsion's parameters on the microstructure and on the resulting drug-release profile, as well as on the physical and mechanical properties. The structure of the semi-occlusive top layer enables control over vapor transmission, in addition to strongly affecting the drug release profile. Release of the analgesic drugs lasted from several days to more than 100 days. Higher organic:aqueous phase ratios and polymer contents reduced the burst release of both drugs and prolonged their release due to a lower porosity. The addition of reinforcing fibers to this layer improved the mechanical properties. Good binding of the two components, PDLGA and collagen, was achieved due to our special method of preparation, which enables a third interfacial layer in which both materials are mixed to create an "interphase". These new PDLGA/collagen structures demonstrated a promising potential for use in various wound healing applications.

1. Introduction

Over the last decades, wound dressings have developed from the crude traditional gauze dressing to tissue-engineered scaffolds. A wide range of wound dressing formats, depending on their function in the wound, are commercially available or have been investigated [1]. A wound dressing should ideally provide an optimal healing environment which enables rapid healing. It should maintain a moist environment at the wound surface, allow gas exchange, act as a barrier to micro-organisms and remove excess exudates [2]. Most modern dressings are therefore designed according to the well-accepted bilayer structure, in order to provide a better healing environment compared to homogeneous films. The upper, dense "skin" layer is designed to control moisture transmission, prevent bacterial penetration and afford mechanical protection to the wound. The lower spongy layer is designed to absorb wound exudates, smoothly adhere to the wet wound bed and accommodate newly formed tissue [3,4]. In this way a dermal skin substitute, such as Integra™, is obtained, rather than a wound dressing which is replaced frequently. Based on these principles, various bilayer structures in which both layers are based on natural polymers [5–8] or synthetic polymers [4] have been designed over the years. Although natural polymers have the advantage of being similar or identical to macromolecules in the body, they undergo rapid in vivo degradation by proteases [9]. On the other hand, dressings based on synthetic polymers do not fully promote cell adhesion and proliferation due to their inherently inert surface chemistry [10], and do not allow smooth adherence to the wound bed, which may lead to bacterial infection.

In contrast, hybrid bilayer wound dressings are very promising, since they combine the advantageous properties of a natural sublayer and a synthetic top layer.

It is important to note that the complex design of integrating two distinct layers into a single structure is challenging, since it necessitates good adhesion between the two layers in order to ensure that delamination does not take place during use.

Controlled release of bioactive agents from wound dressings has also been studied. Much attention has focused on wound dressings that provide an inherent antimicrobial effect by eluting germicidal components in order to prevent bacterial infection [5,12,13]. To date, not enough research has focused on the release of local analgesics, although pain is a common symptom of acute

* Corresponding author. Tel.: +972 3 6405842; fax: +972 3 6407939.
E-mail address: meitalz@post.tau.ac.il (M. Zilberman).

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and chronic cutaneous wounds [14,15]. Pain is considered the most disturbing symptom during healing and has a huge impact on the patient's quality of life. Poor pain management may lead to significant functional and psychological morbidity [16]. Wound pain is related not only to the wound itself, but also to pain during a dressing change. The latter may also harm the vulnerable underlying tissue and increases the risk of secondary contamination.

Analgesics and local anesthetics have proven to be efficient pain relief medications for acute and chronic pain [17]. Ibuprofen is a widely used analgesic that belongs to the non-steroidal anti-inflammatory drugs. In addition to its pain relief effect, its anti-inflammatory effect may promote wound healing by inhibiting the normal inflammatory wound healing phase [18,19]. Encouragingly, recent studies have shown a beneficial effect on wound pain with the use of a foam dressing (Biatain Ibu, Coloplast A/S, Humlebaek, Denmark) that slowly releases low doses of ibuprofen. However, the eluting span of the drug was relatively short and prolonged 2–7 days [17,19]. Bupivacaine hydrochloride is a long-acting local anesthetic which causes reversible nerve conduction blockage by preventing the generation of action potentials [20]. It is commonly used due to its rapid onset and relatively long-lasting anesthetic effect. However, its fast local clearance and high systemic absorption necessitate frequent administration of local doses. A review of the literature indicated that local infiltration of local anesthetics has a detrimental effect on the first two stages of wound healing [21].

We have recently reported the development of bioresorbable core/shell fibers loaded with analgesic drugs [22]. Preparation of the porous drug-loaded shell is based on the freeze-drying of the inverted emulsions technique. That study describes the effects of various formulation and process parameters on the shell structure and on the resulting drug release profile [22]. The drug-loaded porous structure can be used as the top layer in a bilayer wound dressing. In the current study, we developed two such hybrid bilayer wound dressings, based on this concept. Both of them combine a drug-loaded porous poly(DL-lactic-co-glycolic acid) (PDLGA) top layer with a spongy collagen sublayer. Ibuprofen and bupivacaine were incorporated into the top layer for pain management. The top layer can be tailored to produce the desired drug release kinetics and also to control moisture evaporation from the dressing. The spongy collagen layer is designed to maintain high absorption of wound exudates and to accommodate newly formed tissue. Based on our previous study [22], several formulations were selected to serve in the upper layer of the hybrid structures described in the current study. These emulsions were selected based on their stability, resulting microstructure and drug release profiles. The mechanical and physical properties of the hybrid structures are described here, with emphasis on their function as wound dressings as skin substitutes, as well as the drug release profiles from the hybrid structures.

It is important to note that there are no available bioresorbable bilayer dressings which combine the advantages of total biodegradability and intrinsic topical analgesic treatment. In the present paper we also report a simple methodology for integrating the collagen sponge layer with a synthetic PDLGA layer into a unique hybrid structure, and the characteristic features of the newly designed dressings in terms of microstructure, mechanical and physical properties as well as drug release profiles.

2. Materials and methods

2.1. Materials

The following materials were used.

Synthetic polymers: poly(DL-lactic-co-glycolic acid) with a copolymeric ratio of 50% lactic acid and 50% glycolic acid, (50/50 PDLGA), inherent viscosity (i.v.) = 0.4, 0.65 or 0.89 dl g^{-1} (in $CHCl_3$ at 30 °C) (molecular weights (MW) approximately 20, 50, 90 KDa, respectively) (Absorbable Polymer Technologies, Inc., USA).

Reinforcing fibers: PDS* II polydioxanone monofilament absorbable suture with a diameter of 0.20–0.25 mm (Ethicon, Johnson & Johnson, Belgium).

Natural polymer: collagen-klee® 10 × 10 × 0.5 cm (a natural resorbable spongy membrane from porcine dermis consisting of a minimum of 96.75% native collagen type 1) (Medical Biomaterials Products GmbH, Germany (1010S)).

Drugs: bupivacaine hydrochloride (Sigma B-5274), ibuprofen sodium salt (Sigma I-1892). Their molecular structure and properties are presented in Table 1.

Reagent: 1,1,1,3,3,3-hexafluoro-2-propanol (Spectrum Chemical Mfg. Corp. H-1008).

2.2. Wound dressing preparation

2.2.1. Preparation of the inverted emulsion

A known amount of PDLGA was dissolved in chloroform to form the organic phase. A known amount of drug (5% drug w/w, relative to the polymer weight) was dissolved in double-distilled water (DDW) to form the aqueous phase and was then poured into a test tube containing the organic phase. Homogenization of the two phases was performed for 90 s at 16,000 rpm using a Kinematica PT-2500 E Polytron homogenizer.

Three formulations loaded with drugs for pain management were used to produce the top layer.

1. *Reference emulsion:* formulation with 15% w/v 50/50 PDLGA in the organic solution, and an organic-to-aqueous (O:A) phase ratio of 6:1 v/v.
2. *High polymer content emulsion:* formulation with 17.5% w/v 50/50 PDLGA in the organic solution and an organic-to-aqueous (O:A) phase ratio of 6:1 v/v.
3. *High O:A emulsion:* formulation with 15% w/v 50/50 PDLGA in the organic solution and an organic-to-aqueous (O:A) phase ratio of 12:1 v/v.

These three formulations were chosen, based on our previous results that demonstrate their ability to induce distinctly different controlled release patterns [22].

2.2.2. Preparation of the bilayer structures

Two types of bilayer structures were prepared: PDLGA/collagen structure and PDLGA/collagen structure reinforced with fibers. These structures were prepared according to the following procedures.

2.2.2.1. PDLGA/collagen structure. An aluminum tube with rounded and homogenously dispersed holes on its lower surface (diameter D = 5 cm) was developed as a dip-coating instrument. It was connected to a vacuum source which initiated an air flow through the tube that enabled holding the collagen sponge (Fig. 1). The sponge was then dip-coated in a fresh inverted emulsion for a few seconds and then immediately frozen in a liquid nitrogen bath. The samples were then placed in a pre-cooled (−105 °C) freeze-dryer (Virtis 101 equipped with a nitrogen trap) and freeze-dried to preserve the microstructure of the emulsion-based structures. Drying was performed in two stages. The freeze-dryer chamber pressure was reduced to 100 mTorr while the temperature remained at −105 °C. After 5 h a hot plate was turned onto −30 °C overnight. The condenser was then turned off and its plate temperature gradually increased to room temperature while the pressure was monitored between 100 and 700 mTorr. During this step, the