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Porous dressings of modified chitosan with poly(2-hydroxyethyl acrylate) for topical wound delivery of levofloxacin

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ABSTRACT

Absorbable and non-absorbable dressings have been fabricated into sponges via a modified thermally induced phase separation method, using a grafted derivative of chitosan with 2-hydroxyethylacrylate (CS-g-PHEA). The material was synthesized via free-radical polymerization and was characterized with FT-IR and ^{1}H NMR spectroscopies. The swelling ability, biocompatibility and biodegradability of the dressings were evaluated through in vitro assays while antibacterial studies were performed using three different bacterial strains, *Methicillin susceptible Staphylococcus aureus* (MSSA), *Methicillin resistant Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Levofloxacin was used as model drug at different concentrations. Morphological characterization of the drug loaded dressings was performed by scanning electron microscopy, while drug–matrix interactions were evaluated by FT-IR spectroscopy. X-ray diffraction studies were carried out for the identification of the physical state for both neat and drug loaded materials. The prepared dressings showed a significant inhibition zone of the bacteria indicating the antibacterial property of the materials and loaded sponges.

1. Introduction

Skin damages as acute and chronic wounds caused by chronic diseases, burns or post-operative traumas, are of high importance in clinical practice as they are rapidly being colonized by bacteria (Bowler, Duerden, & Armstrong, 2001; Brook, 1995; Culver, Horan, & Gaynes, 1991; Gyssens, 1999; Nichols, 1998; Pruitt, McManus, Kim, & Goodwin, 1998). An intact human skin surface is vital to the preservation of body fluid homeostasis, thermoregulation, and the host's protection against infection, preventing the microbial colonization and the invasion of pathogens into the tissues below the dermis (Robson, 1997). However, due to the skin integration a moisture and warm environment is conduced appropriate for microbial proliferation (Percival & Cutting, 2010). Chronic wounds (Serra et al., 2015) are colonized by both Gram-positive and Gram-negative bacteria (Kim, Lee, & Park, 2014; Storm-Versloot, Vos, Ubbink, & Vermeulen, 2010).

Burn wound infection can inhibit epidermal maturation and results in additional scar tissue formation. Invasion of microorganisms into the tissue layers below the dermis may also cause bacteraemia, sepsis, and multiple-organ dysfunction syndrome. Common microorganisms colonizing open wounds originate from the patient's endogenous skin flora or they can be transmitted from the hospital environment (Pruitt et al., 1998; Serra et al., 2015). The latter tend to be more resistant to antimicrobial agents than those originating from endogenous skin flora. *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the most common agents isolated from chronic wounds, acquired from the patient's endogenous flora and/or an environmental source, are the principal etiological agents of wound infections (Fazli, Bjarnsholt, & Kirketerp-Møller, 2009). In most cases, the bacteria are co-existing complicating the therapy. Furthermore, there is a steadily increasing incidence of infections caused by less commonly encountered microbes, including other Gram-positive and Gram-negative bacteria, fungi, and viruses (Cruse, 1992). Emerging antimicrobial resistance of bacteria wound pathogens, particularly nosocomial isolates as *Methicillin resistant Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci, limits the therapeutic options for the effective treatment of wound infections (Gilchrist, 1994).

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Last studies have proposed that when the amount of bacteria is reduced in the wound, the infection can be more easily handled (Gyssens, 1999). The topical administration of the antibiotics has been evaluated as the most effective treatment for wound infections (Boateng, Matthews, Stevens, & Eccleston, 2008; Gray & Kidd, 1963). Due to the differentiation of the wound types a wide variety of topical wound dressings have been produced in order to deliver active ingredients in targeted sites (Kowalewski et al., 2015; McHugh, Collins, Corrigan, Hill, & Humphreys, 2011). Controlled drug delivery systems in form of dressings provide challenging advantages due to their ability to deliver the antibiotic in a sustained rate without the need for frequent dressing change (Tiwari et al., 2012). Several are the classes of modern dressings such as hydrocolloid, foams and hydrogels. The most promising materials to be used as such dressings are the bioadhesive, synthetic, semisynthetic and natural polymers due to their biodegradability and biocompatibility (Mogoşanu & Grumezescu, 2014; Thomas, 1990). This ability is beneficial as the local concentration of antibiotics is increased while the high systemic dose is avoided (O'Meara, Callum, Majid, & Sheldon, 2000). Recently, a large number of absorbable dressings with haemostatic efficiency have been prepared from natural polymers such as collagen and bovine gelatin (Kim et al., 2005; Hutchinson et al., 2013).

Chitosan (CS), a natural biopolymer soluble in acidic aqueous environment is widely used in such dressings systems due to its low toxicity and its haemostatic ability (Croisier & Jérôme 2013; Huang et al., 2015; Ikeda et al., 2014; Zhang & Ma 1999). Moreover, CS provided bacteriostatic and fungistatic activities. These advantages ranked this bio-polymer as a primordial biomaterial for wound infections management (Li et al., 2012; Wang, Hu, & Cai, 2010). CS and its derivatives were involved in wound infections treatments due to their versatility as drug delivery carriers enhancing the antibiotic and wound-healing effects. Drug delivery vehicles consisting of the above biopolymer have been used for antimicrobial drugs and other drugs (Mpharm, Mpharm, & Chakravarthi, 2010; Noel, Courtney, & Bumgardner, 2008, 2010; Smith, Bumgardner, & Courtney, 2010). In order to produce materials with improved properties, such as increment of water solubility and swelling rate, chemical modification of CS was an effective way to prepare new matrices for medicinal applications (Singh & Ray, 1998). It was reported that hydrophilic segments in hydrophobic polymers strengthen the hydrophobic effect, inducing the solubility rate of the above systems in different pH aqueous solutions (Kakwere & Perrier, 2011; Mun et al., 2008). 2-Hydroxyethyl acrylate (HEA) is the closest analogue of hydroxyethyl methacrylate while poly(2-hydroxyethyl acrylate) (PHEA) can be dissolved in water. Such hydrophilic sponges based on PHEA have been evaluated and show interesting biocompatibility and water sorption ability (Cortazar et al., 2001; Monleo Pradas et al., 2001).

In this work, a grafted chitosan copolymer with PHEA segments (CS-g-PHEA) has been synthesized by free-radical polymerization. CS-g-PHEA porous sponges were prepared using a modified thermal induced phase separation process (TIPS) (Gu, Xue, & Li, 2001; Lloyd, Kim, & Kinzer, 1991). The CS-g-PHEA sponges were further loaded with levofloxacin drug, in order to increase their antimicrobial ability. Levofloxacin (Levo) belongs to fluoroquinolones, approved at 1996 by FDA (Gorman, Samas, & Munson, 2012). Levo is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It has been reported in several researches as antibacterial drug in polymeric delivery systems (Baby, Harsha, Jayaveera, & Abraham, 2012; Cheow, Chang, & Hadinoto, 2010; Li, Wang, Qian, & Shen, 2006; Prabhakar, Chandra Babu, Subha, & Chowdoji Rao, 2013; Qian & Ma, 2004; Shen, Wang, Qian, Liu, & Li, 2004). However, in most of these cases the drug was encapsulated in microparticles or nanoparticles preparing formulations appropriate for oral administration, which could limit its effectiveness in local application. Thus, in the present study a topical delivery of levofloxacin in the form of effective dressings is presented.

2. Materials and methods

2.1. Materials

Chitosan (Medium molecular weight) was purchased from Aldrich Chemical Co. (Stainheim, Germany). 2-Hydroxyethyl acrylate was purchased from Fluka and the hydroquinone inhibitor was removed by passing them, at least twice, through disposable inhibitor–remover packed columns, supplied from Aldrich, before any use. All other reagents and solvents used for the analytical methods were of analytical grade. Levofloxacin API was kindly donated by pharmaceutical company Pharmathen S.A. (Athens, Greece). The liquid media used for in vitro antibacterial assays were nutrient broth (NB), Muller–Hinton broth and Agar (MHB; MHA; Sigma Chemical Co.) and solid media was nutrient agar. Clinical isolates of *Methicillin susceptible Staphylococcus aureus* (MSSA), *Methicillin resistant Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* were obtained from the Laboratory of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Greece and were identified by conventional biochemical profiles according to the CLSI criteria (National Committee for Clinical Laboratory Standards, 2001).

2.2. Synthesis of CS-g-PHEA

The synthesis of CS-g-PHEA was performed via a free radical polymerization technique, similarly to previous reports (Mun et al., 2008; Filippousi et al., 2015). Briefly, 10 g chitosan was dissolved in 400 mL H_2O with 2% (v/v) acetic acid. The mixture was stirred overnight at room temperature. Then, 1.5 g of 2-hydroxy ethyl acrylate and 37.5 mg of potassium persulfate were added to the mixture. The grafting reaction (see Fig. S1) was carried out at 60 °C for 2 h under nitrogen atmosphere. Subsequently, the chitosan solution was washed several times with purified water and freeze-dried under reduced pressure at −60 °C to obtain the cotton-like final product (CS-g-PHEA). The grafted product was further treated with the Soxhlet extraction techniques to removed PHEA homopolymer that was formed during the reaction.

The final grafting percentage (GP, %) of the material was calculated at 60% on the basis of the percentage mass increase of the final product relative to the initial mass of chitosan (M_{in} and M_{fin} denote the mass of chitosan before and after the grafting process, respectively)

$$GP = \left(\frac{M_{fin} - M_{in}}{M_{in}}\right) \times 100\% \quad (1)$$

2.3. Preparation of CS-g-PHEA dressings and levofloxacin loaded CS-g-PHEA

Cryogenic induced phase separation technique was used in order to formulate the dressings. Accordingly, a weighed amount of CS-g-PHEA was dissolved into acetic acid (0.2 mol/L) to form a homogeneous chitosan solution at a concentration of 20 mg/mL. Then, a diluted glutaraldehyde (GLA) aqueous solution (1%, v/v) was added. The mixed solution was poured into a glass tube. The sample was allowed to freeze for 20 min at −10 °C, at which point linear ice crystals extending through the polymer could be seen. After the phase separation, the sample was then freeze-dried (Coolsafe 110-4, Scanvac) for 48 h to get the CS-g-PHEA sponge. Finally, the produced CS-g-PHEA sponge was further dried for another 48 h under vacuum at room temperature to remove the residual water (Gu et al., 2001).