Carbohydrate Polymers 127 (2015) 451–461

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Incorporation of antimicrobial peptides on functionalized cotton gauzes for medical applications

A.P. Gomes [a], J.F. Mano [b,c], J.A. Queiroz [d], I.C. Gouveia [a,*]

[a] FibEnTech – Fiber Materials and Environmental Technologies – Research Unit, Faculty of Engineering, University of Beira Interior, 6201-001 Covilhã, Portugal
[b] 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal
[c] ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
[d] Health Sciences Research Centre, University of Beira Interior, 6201-001 Covilhã, Portugal

ARTICLE INFO

Article history:
Received 29 September 2014
Received in revised form 25 March 2015
Accepted 31 March 2015
Available online 14 April 2015

Keywords:
Antimicrobial peptides
hBD-1
β-Defensin-1
Human
Dermaseptin
Cys-LC-LL-37
Magainin 1
Biocompatibility
Wound-dressing

ABSTRACT

A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from animals and plants during the past two decades. Among them, peptides are the most widespread resulting in a new generation of antimicrobial agents with higher specific activity. In the present study we have developed a new strategy to obtain antimicrobial wound-dressings based on the incorporation of antimicrobial peptides into polyelectrolyte multilayer films built by the alternate deposition of polycation (chitosan) and polyanion (alginic acid sodium salt) over cotton gauzes. Energy dispersive X ray microanalysis technique was used to determine if antimicrobial peptides penetrated within the films. FTIR analysis was performed to assess the chemical linkages, and antimicrobial assays were performed with two strains: *Staphylococcus aureus* (Gram-positive bacterium) and *Klebsiella pneumonia* (Gram-negative bacterium). Results showed that all antimicrobial peptides used in this work have provided a higher antimicrobial effect (in the range of 4 log–6 log reduction) for both microorganisms, in comparison with the controls, and are non-cytotoxic to normal human dermal fibroblasts at the concentrations tested.

1. Introduction

Several authors found that there was a significant absorption of antibiotic, when it is placed directly on the wound as a cream, which may increase the risk of cytotoxicity of the treated tissues, because in this case easily excessive amounts that can be used and it is difficult to control the optimal amount of cream. (Boosalis, McCall, Ahrenholz, Solem, & McClain, 1987; Mi et al., 2002; Wang, Wang, Zhang, Zapatasirvent, & Davies, 1985). Likewise, it is important to develop a method to control the release of antimicrobial agents.

It also has been reported that higher concentrations of some compounds are toxic to tissue and may be a burden to organs or lead to the development of antibiotic resistance (Boateng, Matthews, Stevens, & Eccleston, 2008; Dave, Joshi, & Venugopalan, 2012; Hidalgo & Dominguez, 1998). Compounds most commonly incorporated into dressings to control or prevent infection are silver (Boateng et al., 2008), povidone–iodine (Misra & Nanchahal, 2003) and polyhexamethylene biguanide (Motta, Milne, & Corbett, 2004). On the other hand, semi-solid preparations such as silver sulphadiazine cream (Hudspith & Rayatt, 2004) and silver nitrate ointment (Moir & Serra, 2012) are used to treat bacterial infection on the surface of the wound but direct application onto open wounds can be very painful (Thakoersing et al., 2012) and the scientific evidence for the efficacy of these agents in wounds is scarce. Common topical antibiotics also include mupirocin (Rode, Hanslo, Dewet, Millar, & Cywes, 1989), neosporin (Sinha, Agarwal, & Agarwal, 1997) and tetracycline (Kumar, Bai, & Krishnan, 2004). However, these antibiotics are ineffective when resistant bacteria colonize the wound (Cookson, 1998; Hetem & Bonten, 2013). Moreover, it is important that slow release of antimicrobial agent from wound dressing have the advantage of treating infected wounds in a mild way (Elsner, Berdicevsky, & Zilberman, 2011; Kostenko, Lyczak, Turner, & Martinuzzi, 2010).

Since the beginning of the antibiotic era in the 1940s, the use of antibiotics has resulted in the continual emergence of resistant strains of bacteria, further complicating the clearance of infection in cutaneous wounds (Gibson et al., 2012). Therefore, a new and innovative strategy is needed to combat infected cutaneous

* Corresponding author. Tel.: +35 1917248532.
E-mail address: igouveia@ubi.pt (I.C. Gouveia).

http://dx.doi.org/10.1016/j.carbpol.2015.03.089
0144-8617/

wounds. For this purpose a new strategy foresees the use of antimicrobial peptides (AMPs) as potential antibacterial for wound dressing application (Boateng et al., 2008). AMPs are a potential therapeutic compounds, they are essential components of the human innate immune system and as such contribute to the first line of defence against infections (Nizet et al., 2001; Zasloff, 2002).

AMPs produced in bacteria, insects, plants, invertebrates and vertebrates, are an important component of the natural defences of most living organisms. AMPs exhibit potent killing of a broad range of microorganisms, including Gram-negative and Gram-positive bacteria, fungi and viruses (Dai et al., 2010; Leguen et al., 2007; Marshall & Arenas, 2003). AMPs are diverse in their sequence and structures. They are generally small (10–50 aminoacids) and have at least two positive charges (da Silva & Machado, 2012). Besides antibacterial and antifungal activities, some of AMPs also possess antiviral or anticancer properties. AMPs exert their antifungal or antibacterial effects by interacting and destabilizing the microbial membrane, leading to cell death (Sato & Feix, 2006; Wimley & Hristova, 2011). The exact mechanism by which AMPs exert their antimicrobial properties is yet unknown, but it is generally accepted that cationic AMPs interact by electrostatic forces with the negatively charged phospholipid head groups on the bacterial membrane and cause disruption, resulting in bacterial killing (da Silva & Machado, 2012; Zasloff, 2002).

There are different methods based on physical or chemical immobilization of AMPs to develop antibacterial surfaces. In covalent immobilization the AMPs chemically react with a given surface to form stable antimicrobial coatings (Onaizi & Leong, 2011). Surfaces that are not reactive toward AMPs can undergo some surface treatment to introduce the desired functional groups that will allow the grafting of AMPs in a further step (Banerjee, Pangule, & Kane, 2011). Among the physical immobilization methods Layer by Layer (LbL) can be a promising technique to immobilize AMPs on materials surfaces. This method is based on the alternate adsorption of polycations and polyanions on a solid substratum (Ariga, Hill, & Ji, 2007). In this work AMPs can be simply embedded in the multilayer structure to prepare functional cotton gauzes.

From reports in the scientific literature, a group of 4 AMPs was selected for the present study: hBD-1, β-Defensin-1, human; Dermaseptin; Cys-LC-LL-37 and Magainin 1. All of these AMPs have been described to have an antimicrobial activity against different microorganisms (Guani-Guerra, Santos-Mendoza, Lugo-Reyes, & Teran, 2010; Jiang et al., 2012; Nascimento, Franco, Oliveira, & Andrade, 2012; Nicolas & El Amri, 2009). Another important factor of these AMPs there are cysteine residues, which promote the formation of disulfide bonds in the molecular structure, making them resistant to proteases, temperature and pH (Bulet, Stocklin, & Menin, 2004).

Defensins are cysteine-rich cationic antimicrobial peptides that play an important role in innate immunity they are known to contribute to the regulation of host adaptive immunity and capacity of re-epithelialisation of healing skin (Sakamoto et al., 2005).

Dermaseptin is a linear polyatomic peptide, composed of 34-residue anionic, which are structured in amphipathic α-helices in apolar solvents. Several Dermaseptins have been reported to inhibit the activity of microbial cells, rapidly, efficiently and irreversibly without toxic effects on mammalian cells (Marshall & Arenas, 2003).

LL-37 induces keratinocyte migration required for re-epithelialization of the wound. LL-37 is also an important factor in the proliferation and formation of vessel-like structures, and induces functional angiogenesis important for cutaneous wound neovascularization. LL-37 has antimicrobial activity against both Gram-positive and Gram-negative bacteria, stimulates wound vascularization and re-epithelialization of healing skin and has antitumor activity. The human cathelicidin LL-37 also has been associated with host stimulatory events important to the wound repair process (Izadpanah & Gallo, 2005).

In this work we used a new line of LL-37 from AnaSpec, Inc., Cys-LC-LL-37. This is a new AMP like the LL-37, but has a broad range antimicrobial activity. This new AMP was obtained with one cysteine, where LC is a 6-carbon linker.

Magainin 1 is a 23-amino acid cationic AMP, which has a α-helical structure and is characterized by being a cationic and amphipathic molecule. Magainin 1 reveals multiple functions related to membrane interactions, being active toward multiple pathogens. This peptide also carries a positive net charge at a neutral pH level and has hydrophobic residues that are essential for antimicrobial activity (Nascimento et al., 2012; Speranza, Taddei, & Ovidi, 2007). This AMP has broad-spectrum, nonspecific activity against a wide range of microorganisms, including viruses, Gram-positive and Gram-negative bacteria, protozoa and fungi, may also be haemolytic and cytotoxic to cancer cells and is a bactericide (Zairi, Tangy, Bouassida, & Hani, 2009). These observations suggest AMPs serve a dual role in wound healing: killing bacteria and stimulating complex host repair phenomena.

The biomaterials chosen for the functionalization of cotton gauze were chitosan (CH) and alginic acid sodium salt (ALG), both known as biodegradable, nontoxic and biocompatible polymers. CH is widely used as wound dressings and has been shown to have mucoadhesive properties, cationic nature, anti-bacterial and haemostatic properties (Alves, Picart, & Mano, 2009; Jayakumar et al., 2010).

ALG is known to be nontoxic, having hemostatic action and biocompatible with a variety of cells, ALG has been studied for application as biomaterials and as wound dressings (de Moraes & Beppu, 2013). Due to its properties CH and ALG are already widely used in biomedical applications (Caridade et al., 2013; Lee & Mooney, 2012; Martins, Merino, Mano, & Alves, 2010).

An ideal wound dressing can restore the milieu required for the healing process, while simultaneously protecting the wound bed against bacteria. This has encouraged the development of improved wound dressings that provide an antimicrobial effect by eluting germicidal compounds such as iodine or most frequently silver ions. Such dressings are designed to provide controlled release of the active agent through a slow but sustained release mechanism which helps to avoid toxicity and yet ensures delivery of a therapeutic dose to the wound (Peles, Binderman, Berdicevsky, & Zilberman, 2013).

Based on the previous concept, in this study, we have incorporated AMPs onto a substrate of cotton gauze functionalized with layers of CH and ALG. Functionalized cotton gauzes with CH and ALG were obtained via LbL electrostatic deposition, as described in a work already published (Gomes, Mano, Queiroz, & Gouveia, 2012). The aim of this work is to incorporate AMPs between the layers of CH and ALG. These layers are based on the alternate deposition of oppositely charged polyelectrolyte layers (CH is a polycation and ALG is a polyanion), this deposition was made on cotton gauze.

The embedding of active agents by LbL is a very recent area of research receiving great interest due to the advantage of obtaining control over drug release. Not so recent and with a large number of published papers, LbL was developed for drug delivery systems through microcapsules (Johnston, Cortez, Angelatos, & Caruso, 2006; Quinn, Johnston, Such, Zelikin, & Caruso, 2007; Sukhishvili, 2005; Tang, Wang, Podsiadlo, & Kotov, 2006; Wang, Angelatos, & Caruso, 2008). LbL deposited thin films were first developed by Decher and co-workers (Decher, 1997). They proposed a protocol for the preparation of thin films based on alternate and repeated adsorption of polycations and polyanions on the surface of a solid substrate from solution. A diversity of materials have been employed as building blocks for LbL films, including synthetic polymers, biopolymers, inorganic nanoparticles, etc. (Ariga et al.,