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Electrospun polymeric dressings functionalized with antimicrobial peptides and collagen type I for enhanced wound healing

H P Felgueiras[1] and M T P Amorim

Centre for Textile Science and Technology, Department of Textile Engineering, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal

Email: helena.felgueiras@2c2t.uminho.pt

Abstract. Modern wound dressings combine medical textiles with active compounds that stimulate wound healing while protecting against infection. Electrospun wound dressings have been extensively studied and the electrospinning technique recognized as an efficient approach for the production of nanoscale fibrous mats. The unique diverse function and architecture of antimicrobial peptides (AMPs) has attracted considerable attention as a tool for the design of new anti-infective drugs. Functionalizing electrospun wound dressings with these AMPs is nowadays being researched. In the present work, we explore these new systems by highlighting the most important characteristics of electropsun wound dressings, revealing the importance of AMPs to wound healing, and the methods available to functionalize the electrospun mats with these molecules. The combined therapeutic potential of collagen type I and these AMP functionalized dressings will be highlighted as well; the significance of these new strategies for the future of wound healing will be clarified.

1. Introduction

Wound dressing design and fabrication are important segments of the textile medical and pharmaceutical wound care market worldwide. In the past, traditional dressings were used to simply manage the wound, to keep it dry and prevent bacterial entrance. Nowadays, the fabrication of wound dressings aims to create an optimal environment that accelerates wound healing, while promoting oxygen exchange and intensively preventing microbial colonization [1].

Electrospinning has become one of the most popular processes to produce medical textiles in the form of wound dressings. This is a simple and effective method to produce nanoscale fibrous mats with controlled pore size and structure, from both natural and synthetic origin polymers. This technique has gain much attention because of its versatility, reproducibility, volume-to-surface ratio and submicron range [2-4]. Recently, functionalizing these electrospun wound dressings with active compounds that accelerate wound healing and tissue regeneration has become the major goal [5]. The rising of antibiotic-resistant infection agents has increased the need for such therapies. While antibiotics act selectively against bacteria, dressings functionalized with antimicrobial peptides (AMPs) act at multiple sites within microbial cells, reducing the likelihood of bacteria to develop resistance [6]. The combination of collagen type I (Col I), one of the most important extracellular

matrix (ECM) proteins to wound healing, with these AMP-polymer mat systems has yet to be investigated. Col I has been highlighted as uniquely suited for wound dressing therapies because of its involvement in all phases of wound-healing [7]. Thus the combination of Col I with the AMPs would represent a new step further in the optimization/development of new generation wound dressings.

In the present paper, we reviewed the basic concepts associated with electrospinning technique and explored the use of AMPs in wound healing. Further, we established AMPs functionalization methods and highlighted the importance of Col I to tissue regeneration.

2. Electrospun Wound Dressings

Electrospinning is a cost-effective, simple and straightforward technique, that allows the production of continuous nanofibers with specific properties. Modern dressings are design to facilitate wound healing, protect the wounded site from repeated trauma and prevent infection. It has been established that the ideal dressing should be haemostatic, absorb exudates, maintain moisture balance, protect against pathogens, adapt to the wounded site, cause no pain and be low cost [2-4]. Nanofibrous mats produced by eletrospinning may accommodate all these demands.

2.1. Technique: Principles

Electrospinning is based on the principle that strong mutual electrical repulsive forces overcome weaker forces of surface tension in the charged polymer liquid. It is capable of consistently produce fibers in the submicron range, from 2 nm to several micrometers, with extremely high surface-to-volume ratio, tunable porosity and malleability. The equipment consists of a syringe needle connected to a high DC voltage source of tens of kVs that generates an electrical field with the collecting plate and impels the polymer solution to extrude. As it is released, the polymer solution jet becomes unstable and elongates, subdividing geometrically into nanofiber jets that are collected at an optimal distance. At the same time, the volatile organic solvent used to prepare the polymer solution evaporates. In the end, a mat composed of individual continuous nanofibers is obtained [2-4].

The mat's topography, morphology and fiber orientation are defined by the operating conditions (applied voltage, flow rate, distance to collector), solution properties (polymer concentration, viscosity, solvent volatility, surface tension, conductivity) and environment conditions (temperature, humidity) [8]. By modifying and adapting these parameters to fit our wounds demands, multiple distinct nanofibrous medical textiles can be produced.

2.2. Natural and Synthetic Polymers for Wound Dressings

Natural polymers, which derive from renewable sources, are widely used in medicine regenerative because of their intrinsically bioactive and biodegradable properties and similarity to the ECM. Between the many, polysaccharides, including cellulose, hyaluronic acid, chitin, chitosan, alginates, etc., are the most extensively used to manage and treat wounds. Cellulose is perhaps the most used as it is an abundant polysaccharide based on glucose of particular interest to wound healing due to its flexibility, unique nanostructure and remarkable physical-chemical properties, biocompatibility, biodegradability, hydration capacity and antimicrobial features [9].

Many formulations of individual or blends of synthetic polymers have been investigated for the production of dressings with successful results, including poly(ε-caprolactone) (PCL), polyurethane (PU) and poly(vinyl alcohol) (PVA). PCL is bioresorbable and biocompatible and has been applied for the production of wound dressing since the 1970s [10]. PU is a biodegradable hydrophobic polymer with excellent mechanical properties, high elongation capacity, good abrasion resistance, high flexibility and hardness, and blood compatibility [11]. As a biodegradable, non-toxic or carcinogenic, biocompatible polymer with good mechanical properties, PVA is desirable for wound healing due to its flexibility and swelling capacity in aqueous environments [12].

3. Antimicrobial Peptides

From a microbiological point of view, the primary function of intact skin is to prevent invasion and colonization of potential pathogens. Exposure of subcutaneous tissue, following loss of skin integrity, provides a moist, warm, and nutritious environment for microorganisms to colonize and proliferate. To fight these infections, multicellular organisms have evolved and develop an arsenal of host-defense molecules, the AMPs. These natural and synthetic peptides provide a non-specific defense against a broad spectrum of invaders, such as bacteria, fungi, and certain viruses, acting like a component of innate immunity [5].

AMPs are low molecular weight molecules composed of 5 to 100 amino acid residues. AMPs are often cationic due to the excess of lysine, arginine and histidine amino acids, and most AMPs are amphipatic, containing both hydrophilic and hydrophobic amino acid residues organized in a helical molecule. The amphipatic helical structure is most effective interacting with biomembranes, since it endows the AMPs with the capacity to bind to lipid components (hydrophobic regions) and phospholipid groups (hydrophilic regions) [13]. AMPs can be subdivided in four main classes, according to its structural diversity: α-helix, β-sheet, extended and loop. The α-helix and β-sheet configurations are the most common; the first is formed only when the peptide contacts with a membrane, and the second is stabilized by 2 to 4 disulfide bonds. The less common, extended and loop, display a curved form in response to a simple disulfide bond or the presence of proline residues in its structure. AMPs can also be classified based on their target microorganism: antibacterial peptides (most common), which target the cell membranes disintegrating the lipid bilayer structure; antiviral peptides, which neutralize the viruses by integrating in either the viral envelope or the host cell membrane; antifungal peptides, which kill by targeting either the cell wall or the intracellular components; and antiparasitic peptides, which kill by direct interaction with the cell membrane [6].

3.1. AMPs in Wound Healing

AMPs are found in a variety of tissues. They were first observed in mammalian skin in the form of cathelicidin PR-39 [14]. Later, human cathelicidin hCAP-18 was detected in epidermal keratinocytes. Since then, many others, like the human defensin hBD-3, were studied and cloned for wound healing purposes [15].

The hCAP-18 is the only human cathelicidin. It is found in specific granules of neutrophils, in keratinocytes during skin inflammation or even in the lungs. Plasma contains, as well, a high concentration of hCAP-18 bound to lipoproteins. The LL-37, which is the antibacterial C-terminus of hCAP-18, displays broad antimicrobial activity against both Gram-positive and Gram-negative bacteria, has synergistic antibacterial effects with defensins, and is a potent chemoattractant agent for neuthophils, monocytes and T cells. LL-37 has been shown to be up-regulated in the skin following injury and, thus, to increase its sensibility towards infection [16,17]. The human β-defensin-3 or hBD-3 possesses a broad bactericidal activity against both Gram-positive and Gram-negative bacteria, and has also been associated with increased cytokine secretion and keratinocytes expression, cell migration and proliferation, enhanced anti-inflammatory activity, and accelerated wound closure [18].

Aside from human-derived AMPs, there are others from amphibian, vertebrate, insect or pathogen origins that have revealed great results as wound healing promoters. The isolation of magainins from *Xenopus laevis* species led to the discovery of a wide range of amphibian peptides of great potential. Pexiganan, for instance, was one of the first AMPs to be synthesized from mangainin. It is mainly applied in the treatment of infected wounds, including diabetic ulcers, and has been shown to reduce microbial burden. Pexiganan is also known to stimulate migration of cells involved in tissue reconstruction [19].

3.2. AMPs Functionalization

Following concerns over the development and spread of antibiotic-resistant strains of bacteria, the use of dressings that combine AMPs with well-established biomedical polymers has increased. Functionalizing AMPs onto electrospun dressings has become a most important process [20].

There are many strategies used to immobilize AMPs onto electrospun dressings. The most common and simplest is the co-spinning method. Here, AMPs are immobilized as the polymeric nanofibers are produced. Multifunctional bioactive nanofibrous dressings, with an all-in-one approach, are therefore the result of co-spinning [21]. Physical adsorption methods, which include adsorption and layer-by-layer assembly, involve physisorption of AMPs through non-covalent or multidentate interactions at the electrospun surface. Adsorption is the most straightforward approach. It requires only the immersion of the electrospun mats in a solution containing the selected AMPs, for the time required to reach saturation. This method, however simple, is most challenging without using binding agents, as quick desorption of the AMPs in response to mechanical forces remains a complication. In the layer-by-layer approach, AMPs are sandwiched between two polyionic polymers. Using this method a flexible number of layers with controlled AMP loading can be prepared by exchanging electrospun solutions at the appropriate time. As a downside, the AMPs immobilized in between layers may have more difficulties to diffuse towards the wounded site [22].

Compared to physical immobilization, covalent binding offers many more advantages, including minimizing AMPs leaching, providing long-term stability and lowering toxicity. It can be accomplished by two ways: "graft to" approach, which involves the covalent coupling of the intact AMPs to the electrospun surface; or "surface initiated" strategy, which requires the synthesis of the AMPs from initiators or spacers bearing reactive groups covalently immobilized onto the mat's surface. "Graft to" strategies entail the activation of the electrospun surfaces to generate free binding groups like amines, carboxylic acids, aldehydes or thiols. More often than expected, the linkage between these free functional groups and the AMPs may be hydrolyzed or broken by mechanical forces, even though the covalent bonds created are strong and irreversible [20]. Polymer resins like polyethylene glycol (PEG) are frequently functionalized onto electrospun dressings to induce "surface initiated" immobilization. As a linker, PEG aside from reducing bacteria interactions also allows rapid and free orientation of the bound AMPs at the interface, thus enhancing the protective performance of the bound AMPs and its action mode [23].

4. Collagen Type I in Wound Healing

Collagen is an extremely important ECM protein, majorly present in the connective tissues (i.e. tendons, bone, skin, etc.). 25-35% of the total amount of proteins in the human body are collagen and, from the 16 existing types, Col I is the most common, representing near 90% of the organic mass of bone [24]. Col I has an excellent biocompatibility, which makes it popular in artificial tissue and wound dressings manufacture. The native fibrillar collagen is the most important polymorphic form of collagen, which makes manufacture of fibrous dressing products possible [25]. In the form of wound dressings, Col stimulates the wound healing cellular and molecular cascades, development of new tissues and wound debridement [26]. In fact, Col I has been highlighted as uniquely suited for wound dressing therapies because of its involvement in all phases of wound-healing. Platelets aggregate around exposed collagen and secrete factors that stimulate the intrinsic clotting cascade responsible for a stable hemostatic "plug". Further, collagen-based wound dressings have been shown to address the issue of elevated levels of matrix metalloproteinases, a key component of chronic wounds known to degrade non-viable and viable collagen, by acting as "sacrificial substrates" in the wound [7]. Collagen fibrils have been combined with other polymeric matrixes to produce dressings with the ability to absorb wound exudates to maintain a moist environment and, thus, stimulate wound healing [27,28].

5. Conclusions and Future Perspectives

Due to the continue rising of antimicrobial resistant pathogens, the need for engineered alternated treatments for acute to chronic wound care has increased. As a first strategy to overcome this issue, AMPs have been loaded onto existing textile medical dressings to improve their healing and antimicrobial capacities. We highlighted the most well known AMPs and the most appropriate methods to functionalize the surface of electrospun mats with such molecules. This is still a very new formulation and further research should be conducted. Indeed, long-term therapeutics using AMPs

functionalized dressings should be carefully evaluated to prevent the risk of compromising our innate immune defense and, therefore, the ability to control commensal microbiome and microbial infections. Functionalizing surfaces with AMPs should be managed by standardized tests that not only evaluate the action of the AMPs but as well its stability, releasing abilities and tunable performance. The level of control in peptide loading and release timescales that are required in applications that could benefit from such antimicrobial profile has thus far not been demonstrated. Because they are still being developed and tested, these systems, AMPs-polymeric mat, should be cautiously defined so that the best combination between selected polymer, mechanism of action, AMPs and immobilization process is achieved. Although Col I has been extensively used in wound healing and its potential already demonstrated, the combination with AMPs-polymeric mats systems has yet to be explored. In a near future, we intend to examine the synergistic performance of these molecules in the treatment of chronic wounds, namely diabetic ulcers. We are confident these new systems aside from acting against the pathogens will also accelerate the wound healing process by establishing a symbiotic action.

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