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Coaxial Wet-Spun Fibers Loaded with Enzyme-Inhibiting Peptide for Chronic Wound Care †

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Abstract: Chronic wounds (CW) are a worldwide concern and play a great impact on the patients' quality of lives. The tetrapeptide Ala-Ala-Pro-Val (AAPV) has proven to be an effective inhibitor of the enzyme human neutrophil elastase (HNE), which reaches abnormally high rates during inflammatory processes. Nevertheless, such peptides usually present low stability in physiological media. One alternative to solve that problem is to incorporate the peptides within polymeric structures (e.g., coaxial fibers). This way, not only is the peptide protected from the surrounding environment, but also a controlled and sustained release of the peptide can be achieved. To this end, coaxial wet-spun fibrous structures loaded with the AAPV peptide were proposed, working as a delivery system for combating infections and restore local enzymatic activity to normal levels.

Keywords: chronic wound care; drug delivery; therapeutic tetrapeptides

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1. Introduction

CW are usually associated with malfunctioning cell matrices, high microbial counts and prolonged inflammatory responses, resulting from gradual tissue degradation in which proteolytic enzymes act against endogenous and growth factors, compromising their integrity. As soon as microorganisms colonize the wounds, neutrophils liberate proteases (e.g., HNE), which continue degrading the healthy elements that compose the wound bed and increase their chronicity. As a result, CW are unable to heal in an appropriate period of time, as they do not progress through the stages of healing [1]. Research has shown that 1 to 2% of the developed countries' population will experience at least one CW in their lifetime, showing that CW prevalence and its effects should not be underestimated [2,3].

Fiber-based dressings consist of a promising alternative for the treatment of this type of wound. Such dressings can be produced by spinning techniques and present good tenability, high surface area and tunable porosity, limiting the penetration of external pathogens. Moreover, this type of dressings displays good hemostasis, ensures a moist environment, a high absorbance capacity and oxygen permeability, and contributes to a faster skin regeneration [3,4]. Electrospinning has been commonly applied for the production of such type of dressings. However, the produced mats frequently present high fiber packing density and small porosity, which impose significant restrictions for cell infiltration

throughout the innermost regions of the scaffold and for the maintenance of micro-environments that resemble in vivo natural interstitial fluid conditions [5]. The wet-spinning technique has arisen as an alternative technique, by being capable of generating hybrid structures with different levels of organization, as well as chemical and physical properties that facilitate cell infiltration and mimic the dressing environment to actual physiological conditions [6,7]. Likewise, wet-spinning allows for limitations related to polymer thermal degradation or the difficulties in establishing optimal processing parameters, to be circumvented [8,9]. The wet-spinning technique is based on the non-solvent-induced phase inversion process, in which a polymeric solution is extruded into a coagulation bath of a poor solvent or a non-solvent of the processed polymer, precipitating the continuous polymer filament that solidifies into a fiber. As a result, fiber constructs with diameters averaging tens to hundreds of micrometers are produced (nanofibers are less common in wet-spun constructs but still feasible) [10].

In this research, the production of coaxial wet-spun fibers is proposed. The goal is to produce a porous, interconnected structure, modified with specialized biomolecules, for the treatment of CW. The inner layer (core) will be formed of PCL, which consists of a synthetic polymer endowed with excellent mechanical properties, intended to maintain fibers' structural integrity, combined with AAPV, a tetrapeptide that inhibits the action of human neutrophil elastase (HNE), an enzyme that increases its presence by 10–40 times its normal range during skin chronic inflammatory events (usually triggered by microbial infections) [1,11]. The outer layer (shell) will be composed of blends of SA, a biopolymer with excellent swelling properties, with the main purpose of maintaining a moist environment at the wound site, and NCMC, a modified version of chitosan, soluble at neutral to basic pH, also possessing antibacterial activity.

This way, coaxial wet-spun fibers can be achieved, working as controlled release platforms for AAPV and NCMC. With this investigation a step further can be taken in establishing wet-spun constructs for drug delivery in CW care.

2. Wet-Spinning

The wet-spinning technique, a method developed to produce natural and synthetic fibers, was firstly used in the late 19th century. The process consists in the injection of a solution, of a dissolved macromolecular material, through a spinneret directly into a coagulation bath (Figure 1). Here, a polymeric solution can be extruded towards a coagulation bath containing a non-solvent or a poor solvent of the polymer, but miscible with the solvent of the polymeric solution. Thus, as the solvent is removed from the polymer blend, while it contacts with the bath, the fiber solidifies as precipitation takes place [12].

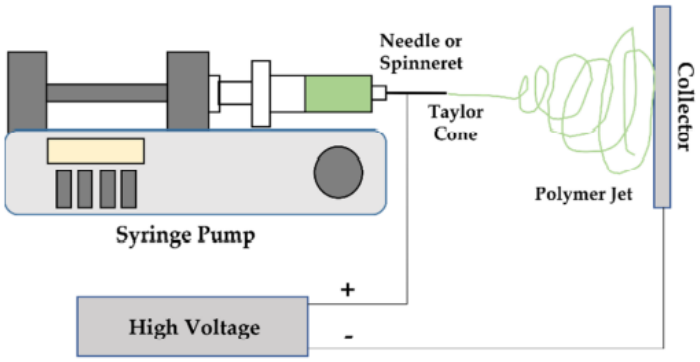

Figure 1. Schematic representation of the wet-spinning process (adapted from [13], with permission from Creative Common CC BY license).

This spinning technique is capable of processing polymers that cannot be melted or heated at high temperatures (a requirement of the melt spinning approach). Further, by

resorting to solvents to prepare polymeric solutions, viscosity limitations can be circumvented [14,15]. Typically, the resulting fibers present large diameters and high porosity and can generated scaffolds with an interconnected open pore structure, desirable for cell penetration, adhesion and proliferation [16]. Moreover, this technique can lead to the production of fibers with unique properties and a wide variety of cross-section shapes and sizes, depending on the type of spinneret employed, the composition of the coagulation bath, drying temperature and the concentration of the polymeric solution [12].

The coaxial wet-spinning method is frequently employed to produce fibers with a core-shell structure. Here, two polymer solutions can be injected concomitantly using a co-axial spinneret and co-extruded into a coagulation bath. More specifically, the setup for this method includes two injection syringes and pumps, connected to two ports, a coagulation bath and a stretching collector [12,13]. Coaxial wet-spun fibers have been applied for several purposes, including not only in the biomedical field, for controlled release and tissue engineering applications, but also for electronic textiles, sensors and actuators [13].

3. Antimicrobial Peptides

Antibiotics have been commonly applied for the treatment of several infections. However, nowadays, this treatment option has been facing many problematics due to the increased resistance of pathogens. Therefore, it is crucial to pursue other options, such as antimicrobial peptides (AMPs).

AMPs consist of host-defense molecules expressed by multicellular organisms intended to control microbial proliferation and modulate the immune response [17]. AMPs are usually low molecular weight molecules, composed of up to 100 amino acid residues, with an overall positive charge [1]. AMPs display a distribution of basic amino acids and hydrophobic residues, aligned in three dimensional formations on opposing faces, resulting in water soluble structures with positive charges and hydrophobic nature. Typically, AMPs are only effective towards one major class of microorganisms, for example bacteria or fungi [18]. Nonetheless, some AMPs can display action against several types of microorganisms and have different modes of action. One example is indolicidin that kills bacteria, fungi and the human immunodeficiency virus (HIV) [19]. The main target of AMPs is the lipopolysaccharide layer of the cell membranes. The binding of AMPs to bacterial membranes causes non-enzymatic disruptions and the selectivity of the binding is dependent on the microbes and their membrane composition. Some AMPs disrupt the bacterial membranes by enzymatic digestion. In addition, some peptides have been reported to being able to cross the lipid bilayer without causing damage, despite killing the bacteria and inhibiting intracellular functions. Moreover, AMPs can also inhibit biofilm formation and disrupt existing biofilms [20].

AAPV has been proven effective in inhibiting the action of human neutrophil elastase (HNE), which is a relevant enzyme involved in immune processes and extracellular matrix (ECM) remodeling during wound healing. In normal conditions, the proteolytic activity of HNE is controlled effectively by endogenous inhibitors. However, in the case of inflammatory processes related to CW, the levels of HNE are abnormally high, reaching 10–40 times the amount registered in acute wounds, being thus responsible for an abnormal degradation of endogenous and supplemental growth hormones [1]. Therefore, AAPV has been considered a competitive inhibitor of HNE for the treatment of chronic skin inflammatory diseases, such as psoriasis. Even though the antimicrobial potential of AAPV has not been disclosed, it is still considered an AMP for its proven host-defense mechanisms associated with enzymatic overexpression inhibition [21].

4. Conclusions and Future Perspectives

Wet-spinning technique constitutes a promising alternative for tissue engineering and drug delivery applications, due to its simplicity and ability of producing fibers with interconnected pore structures and a wide range of diameters. The envisioned coaxial wet-spun fibers containing AAPV present the potential to work as controlled release platforms for regulating local enzymatic activity and fight bacterial infections. If successful, this research can result in an advance in the establishment of wet-spun fibrous structures for wound care.

Solution and processing parameters must be optimized, tuning the mechanical and chemical properties of the intended coaxial fibers, as well as the evaluation of cells' viability and metabolic activity.

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