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# Biodegradable wet-spun fibers modified with antimicrobial agents for potential applications in biomedical engineering

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**Abstract.** Wet-spinning is a non-solvent induced phase inversion technique that allows the production of continuous polymeric microfibers, with a uniform morphology, based on the principle of precipitation. It allows the production of 3D fibrous constructs with an intricated architecture that facilitates cell infiltration, something that is very limited in electrospun nanofibrous mats, thus increasing its interest in biomedicine. Wet-spun scaffolds are also more easily processed and can be loaded with a variety of biomolecules of interest. Antimicrobial agents that display a broad spectrum of activity against bacteria, fungi and viruses have been combined with such constructs demonstrating great potential to fight infections. In the present work, we explore the use of wet-spinning to process both natural and synthetic biodegradable polymers in the form of microfibers, and the necessary processes to modify their surface to increase their antimicrobial profile. The synergistic potential of specialized biomolecules within wet-spun fibrous architectures are also highlighted.

## 1. Introduction

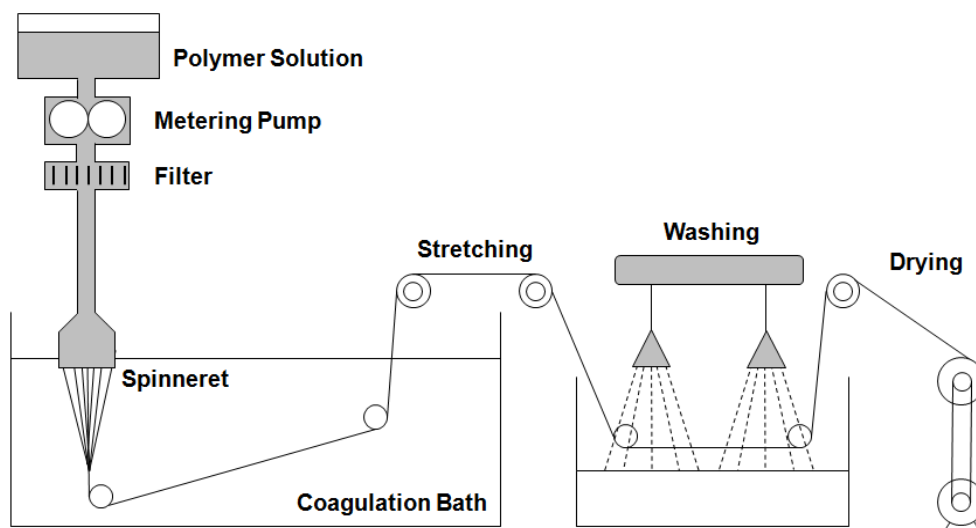
Advances in polymer processing technologies have allowed for new 2D and 3D fibrous constructs to occur and be manipulated as required. With these techniques at our disposal, it is now possible to control the scaffolds architecture, their interconnected network and overall surface chemistry through the incorporation of desirable functional groups. Through those, active biomolecules can be loaded to regulate cell behavior or fight microorganisms' colonization [1].

Several techniques have been used in the development of such constructs, with a variety of macro-shapes and micro- and nanostructures, including drawing, melt blown, template synthesis, phase separation, self-assembly and spinning methods. The drawing method can produce individual, long nanofibers. However, its application is limited to viscoelastic materials capable of tolerating significant deformations. Melt blown also requires thermoplastic polymers and adequate air pressure. Using template synthesis, solid/hollow, individual nanofibers can be produced. Yet, this too presents disadvantages, namely the discontinuous nature of the obtained fibers. Phase separation is a time-consuming technique that comprises stages of dissolution, gelation, solvent extraction, freezing and drying, leading to the formation of non-porous foams. Self-assembly is as well a time-consuming approach, in which units are arranged in specific configurations to attain particular functions in the final product [2, 3]. Polymeric fiber meshes formed of micro and nanofibers produced from spinning techniques display high surface area and increased interconnected open pore structure that facilitates cell migration and infiltration, nutrients and mass transport, and the controlled release of loaded



bioactive agents [1, 4]. Even though the electrospinning technique has been widely pursued in biomedicine in current years, the small porosity and high fiber density that characterizes the produced mats has been highlighted as a negative aspect by limiting cell infiltration within the innermost regions of the scaffolds and/or by being unable to generate dynamic micro-environments that closely resemble the natural interstitial fluid conditions *in vivo* [5]. These features, however, can be achieved using the wet-spinning technique (Figure 1) [4, 6]. With wet-spinning it is possible to generate hybrid structures with different levels of organization and particular arrays of chemical and physical properties, avoiding the problems associated with thermal degradation (melt-spinning) or inability to establish optimal processing parameters [2, 7-9]. In this technique continuous micrometric polymeric fibers can be extruded towards a non-solvent coagulation bath for filament solidification. Wet-spun fibrous constructs can be manufactured with a variety of configurations. The most common are the continuous fiber constructs. However, core-shell or hollow fibers are also very common in drug delivery systems. These are commonly produced via an air-gap wet-spinning system which uses a two-coaxial-nozzle extrusion apparatus in which air flows through the inner nozzle [10]. Wet-spinning has been mostly used in the processing of natural origin polymers, including chitosan, starch, collagen and silk fibroin. However, their potentialities as drug-loading therapeutic delivery systems or tissue engineering scaffolds have extended their uses to other synthetic, biodegradable polymers, namely polycaprolactone (PCL) or poly-L-lactic acid (PLLA) [11].

The employment of antimicrobial agents, like antibiotics, proteins, nanoparticles, or even natural extracts, in biomedical applications is not new. Indeed, many formulations have been proposed with exceptional results. Yet, the contributions of such molecules within the overall properties of wet-spun microfibers or 3D constructs for biomedicine are not entirely clear. In the present work, we explore this further and provide examples of the most successful applications of these modified systems. Special attention will be given to the types of antimicrobial molecules employed in biodegradable constructs and their mechanisms of action.



**Figure 1.** Schematic representation of a wet-spinning apparatus.

## 2. Antimicrobial agents: types and properties

Incorporation of biological cues or biomolecules within the surface or structure of wet-spun fibers has been one of the most resorted strategies to broaden the use of biodegradable polymeric fibrous constructs in biomedical engineering [1, 6, 12]. Between the available options (antibiotics, proteins, peptides, growth factors, natural extracts, etc.), the antibiotics can be highlighted as the most frequently used. These chemical compounds have been around since the 20's with the discovery of penicillin [13]. Antibiotics can be obtained from microbial fermentation or be chemically synthesized. They target

bacteria via their membrane structure or cell wall organization, or by disrupting the functions of specific intracellular components, namely proteins, DNA and RNA, etc [12]. In general, antibiotics represent a primary source of treatment for infections. However, the unchecked and misuse of antibiotics has increased tolerance and led to the emerging of antibiotic-resistant microorganisms [14]. For that reason, alternative biomolecules are now being examined and their synergistic effect with polymeric scaffolds are researched. Natural extracts are an important source of drugs in traditional medicine and are growing in importance each day due to their reduced impact in the environment and extended therapeutic potential. Via their biochemical pathways or secondary metabolites, natural extracts can generate specific chemical responses when facing environmental threats. This way being capable of protecting the plant by acting as antioxidants, free radical-scavengers, UV-light absorbents and antiproliferative agents, which attack microorganisms. The antimicrobial action of natural extracts includes enzyme inhibition, non-specific interactions with proteins, intercalation of pathogenic DNA, and interference with the cell membrane by forming ion channels or inhibiting the adhesion of microbial proteins. Essential oils (EOs), for instance, are volatile compounds that present a strong odor. They exhibit antibacterial, antiviral and antifungal properties associated with analgesic, antiseptic, anxiolytic, anti-inflammatory and antioxidant effects [12, 15, 16]. Proteins, peptides, and growth factors have long been proposed as bioactive molecules to induce specific cell functions due to their biocompatible nature, presence in the human body, and versatile structure from which self-assembly of biomaterials with hierarchical 3D architectures is possible. These are most frequent in tissue engineering to promote an implantable biomaterial recognition and to accelerate integration. Presently, in this category, antimicrobial peptides (AMPs) are the ones with the most potential to fight infections. AMPs act by targeting the lipopolysaccharide layer of pathogens, which is exclusive to them. They exhibit a broad spectrum of activity, with their mechanisms of action being mostly directed towards the cell membrane and/or the intracellular components after membrane penetration [1, 6, 12, 15].

### 3. Biofunctionalized, biodegradable wet-spun fibers

Many biodegradable polymers and hybrid structures have been processed by wet-spinning with exceptional capacities for drug-delivery systems. Most of those resort to the direct processing of the polymer solutions in a coagulation bath containing the bioactive agent or drug to be loaded. Denkbaşı *et al.* proposed the production of chitosan microfibers extruded at 0.1 mL/h within a bath containing different concentrations of the hydrophilic anticancer drug 5-fluorouracil. The formed chitosan filaments were entangled with each other originating scaffolds. Glutaraldehyde was used as cross-linking to maintain the fibers structure and, this way, guarantee the incorporation of the drug. The facile modification method allowed the fibers to release high amounts of the drug in the initial stages of interaction (first 90 min) and, then, to guarantee a continuous but slower release rate for prolonged periods of time. Depending on the cross-linker density applied to the chitosan fibers, much slower release profiles were attained [17]. Yet, there are alternatives in which the biomolecule is incorporated within the polymeric matrix before or after extrusion or is encapsulated in a core-shell structure. For instance, Gao *et al.* suggested the addition of 5-fluorouracil to PLLA wet-spun fibers by homogenous dispersion within the polymeric blend. The drug was initially crushed by fluid jet mill and then dispersed in a chloroform solution containing 1% Span 80 to form a stable suspension. PLLA was then combined and the solution continuously stirred until the fibers were extruded at a fixed flow rate and 25 cm distance from the spinneret. Drug release kinetics was controlled by optimizing the drug content, polymer concentration, non-solvent bath, and extrusion flow rate. Since most of the 5-fluorouracil was incorporated within the PLLA fibers, the initial release burst was not toxic to cells, allowing the drug to be delivered at smaller doses for longer periods, desired for cancer treatments [18]. In a different study, collagen was allowed to self-assemble within a pre-formed chitosan-tripolyphosphate microfiber mesh. After microfiber production, a collagen solution was left to self-assemble into nano and microfibers, subsequently freeze-dried, in order to generate a multi-size architecture. The engineered biodegradable polymeric scaffold revealed exceptional cell activity and cytocompatibility towards fibroblasts and osteoblasts, owing the observed attachment and proliferation rates to the presence of the bioactive

collagen [19]. Hollow microfibers are mostly attractive for tissue engineering of small caliber blood vessels. PCL and PCL/poly(lactide-co-glycolic acid) (PLGA) hollow fibers have been produced via wet-spinning with excellent mechanical resistance and enhanced capacity to transport substances. Here, a spinneret with an outer and inner needle was employed, extruding the polymer solution at a flow rate of 1 mL/min. Distilled water was used as the bore liquid, which was extruded at 2.4 mL/min, and ethanol was used in the coagulation bath. Existing traces of the polymer solvents were then eliminated by placing the fibers in a water bath and exchanging the media periodically. Even though the hollow fibers purpose was the transport of blood components, the incorporation of bioactive or antimicrobial agents at their core was determined possible [20, 21].

Accounts on antibiotic loading onto wet-spun fibers are very frequent. Puppi *et al.* reported on the functionalization of 3D PCL meshes with the fluoroquinolone antibiotics enrofloxacin, active against osteomyelitis pathogens, and levofloxacin, a broad-spectrum antimicrobial biomolecule proven effective against infections of the respiratory and genitourinary tract, skin and skin structures, and osteomyelitis. Here, the antibiotics were loaded within the polymeric matrix and blended continuously until a homogeneous dispersion was attained. Fibers were wet-spun at a control flow rate set at 2.25 mL/h, through a 0.4 mm inner diameter needle into an ethanol coagulation bath (24 h immersion). Loading efficiency was not evidenced as only 18-27% of the drugs were found on the fibers. Regardless, the loaded fibers demonstrated a fast-initial burst release, followed by a sustained liberation of the antibiotics up to five weeks. Cytocompatibility experiments revealed the formation of a cellular layer within the scaffolds after 14 days of culture [5]. To overcome the loading limitations, they proposed a computer-aided wet-spinning approach using a three-arm branched PCL that did not require fiber post-processing. Levofloxacin loading was efficiently controlled to guarantee a prolonged, sustained-release without interference in the microfiber's morphology. The drug was blended with the polymer for 2 h. By using a programmable syringe pump, the blend was then injected at a controlled feeding rate directly into a coagulation bath of ethanol. Even though encapsulation efficiency remained within equal ranges,  $\approx 16\%$ , near 90% of the total drug release was reached *in vitro* over a 5-week period without aggressive initial bursts [22]. In another strategy, Aksoy *et al.* resorted to the encapsulation of the antibiotic vancomycin within microspheres of gelatin, which were prepared by coacervation technique. Briefly, the microspheres were produced by dropwise addition of gelatin containing the vancomycin into corn oil and cross-linked with glutaraldehyde. Then, they were added to PCL and mixed for homogeneous dispersion before being extruded through a 0.5 mm diameter needle into a 4°C ethanol coagulation bath under continuous stirring. They established that the presence of PCL retarded the released of vancomycin from the microspheres, providing a long-term sustained liberation of the drug, with strong antimicrobial action against both *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria [23].

Wet-spun scaffolds of chitosan have been examined for the liberation of both the antibiotic gentamicin and the protein bovine serum albumin (BSA). The goal was to engineer a scaffold with a double function of support material for defect sites and delivery platform of bioactive molecules. Porous, microfibrinous scaffolds were produced from chitosan solutions and used as bare or coated with alginate to serve as contributing layer to facilitate the loading and release regulation of the protein and the antibiotic. Chitosan blend was injected at a speed of 5 mL/h into a bath of Na<sub>2</sub>SO<sub>4</sub> (0.5 M) and left immersed overnight for complete fiber formation. Alginate was then coated onto the fibrous scaffolds via vacuum-pressure cycling. The biomolecules were loaded via a series of vacuum-pressure cycles or blended with the polymers prior to wet-spinning and alginate coating. Data demonstrated the release from the scaffold was facilitate by the absence of the alginate layer; however, in the cases where the alginate coating was present, a decreased initial burst release of BSA and retarding effect of gentamicin was detected. Moreover, release of the agents was controlled via diffusion mechanism, with the size of the biomolecules determining their release rate from the scaffold (BSA was slower than gentamicin due to its larger molecular weight, Mw) [24]. BSA release kinetics has also been examined on PLLA and PLGA wet spun microfibers. Indeed, Lavin *et al.* using a cryogenic emulsion approach encapsulated insulin, lysozyme and BSA within wet-spun fibers of  $\approx 100 \mu\text{m}$  in diameter. Again, protein loading influenced the fibers mechanical strength and conditioned the biomolecules release kinetics in a Mw-

dependent manner. For instance, BSA was found to increase the tensile strength and the elongation at break of the PLGA fibers twofold and fourfold above those of BSA-loaded PLLA, respectively. Still, in both cases, prolonged protein release, up to 63 days, was observed [25]. These studies emphasized the critical role of the molecular weight and chemical structure of the loaded biomolecules within the overall performance of the scaffold. Further, they demonstrated their synergistic effect and the ability to produce sequential delivery systems based on such combinations. 3D constructs loaded with a sequential growth factor release profile have been generated. Fiber meshes were produced from chitosan and from chitosan-polyethylene glycol (PEO) by wet-spinning using  $\text{Na}_2\text{SO}_4$  (0.5 M), NaOH (1 M) and distilled water (3:1:6 v/v) as coagulation bath, in which they were kept overnight, and then combined with nanocapsules of PLGA containing the bone morphogenetic protein 2 (BMP-2) and with nanocapsules of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) containing BMP-7. Nanocapsules were prepared by double emulsion-solvent evaporation technique and were incorporated during production by blending with the polymer solutions or after production via surface-modification with a series of vacuum-pressure cycles. This combination of polymers allowed the premature release of the BMP-2 and the retarded release of the BMP-7. The effect of the delivery agents was more important on the surface-modified fibers, with the BMP sequential delivery raising the levels of the alkaline phosphatase activity per cell. Data reported on the potential of the combinatory effect of polymers and bioactive agents for bone tissue engineering [26].

More recently, as antibiotic-resistant bacteria are becoming a global concern, alternatives for the delivery of antimicrobial agents are being engineered. Natural extracts are becoming more attractive for their reduced impact in the environment and diverse potential as antimicrobial, antiseptic, analgesic, and/or anti-inflammatory agents. In light of this phenomenon, Felgueiras *et al.* proposed the modification of wet-spun microfibers made of cellulose acetate (CA) and PCL with ampicillin (control) and three EOs, cinnamon leaf oil, clove oil and cajuput oil. Microfibers were wet-spun towards an ethanol-based coagulation bath at a constant speed of 0.5 mL/h. EOs were incorporated within the fibers by physical adsorption during a 72 h immersion period at room temperature and constant stirring at 200 rpm. Acquired data reported the ability of the modified fibers to effect on the viability of *Staphylococcus aureus* and *Escherichia coli* bacteria, even at small, immobilized concentrations. Further, the EOs-modified fibers were seen to kill bacteria more quickly and to disrupt the bacteria cytoplasmic membrane more easily than the antibiotic, attesting to their potential to replace antibiotics and to be incorporated within scaffolding materials for applications in which bacterial infections are a target [27]. Overall, the contribution of biodegradable, micro-structured, wet-spun platforms for the delivery of biomolecules of interest was confirmed and their potential for biomedical applications demonstrated.

#### 4. Conclusions

The resourcefulness of the wet-spinning technique, in polymer selection, loading methodology and biomolecule, and fiber morphology (porosity, shape and size), has led to an increase amount of reports on the abilities of biodegradable microfibrinous constructs, originated from natural and synthetic resources, for biomedical applications. 3D wet-spun architectures with a pre-established external shape and macropores internal structure can now be tailored to fit a desirable need. Further, biofunctionalization via biomolecule loading has increased their effectiveness in various areas of biomedical research, turning these systems very promising, and opening new opportunities to integrate such microfibers and scaffolds to currently unexplored areas of tissue engineering.

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