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Review Article

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Biomaterials from Chitosan Processed by Electrospinning

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Abstract

This article provides an overview on the development of electrospinning applied to synthetic and natural polymers. We discussed specifically the application allowing to produce chitosan mats for tissue engineering and wound healing.

Keywords

Biomaterial, Electrospinning, Chitosan, Nanofibers, Wound healing, Tissue engineering

Introduction

The purpose of our work was producing nanofibers made of pure chitosan for pharmaceutical and biomedical applications using electrospinning. A nanoporous mat composed of those fibers is a porous hydrophilic support being used for cell cultures. The advantage of this technique is to obtain nanoscale fibers with high surface area to volume ratio. The electrospinning technique is well adapted to nanofibers production with controlled diameter and composition.

In the following, this process is described and the most important parameters involved were discussed. At the end, the conditions for obtention of chitosan nanofibers were proposed.

Electrospinning

One of the research activities of the Laboratoire Rhéologie et Procédés, LRP, of Grenoble-Alpes University focuses on the elaboration of non-woven nano-fibrous materials via electrospinning. This polymer processing under high electric field can produce in a continuous way a network of ultra-thin fibers with a submicron diameter. Under the action of a strong electric field (involving voltages of up to several tens of kV), a jet of polymer solution is emitted from the apex of the hanging drop (Figure 1A). Surface-charged, this jet is accelerated and stretched (Figure 1B). The solvent evaporates in the first centimeters of propagation of the jet in the air, leaving space for a nanometric fiber polymer collected on a support (Figure 1C).

Electrospinning is a versatile technique but it needs to control a broad number of parameters that belongs to both the polymer processing and the polymer solution properties. Regarding the polymer processing, we can mention the applied voltage and the tip to collector distance that influence the electrical field and consequently the fiber morphology, the shape of the collector that

control the network morphology, the shape of the spinneret that influence the initial shape of the jet, the solution flow rate that has to be adjusted to maintain the Taylor cone.

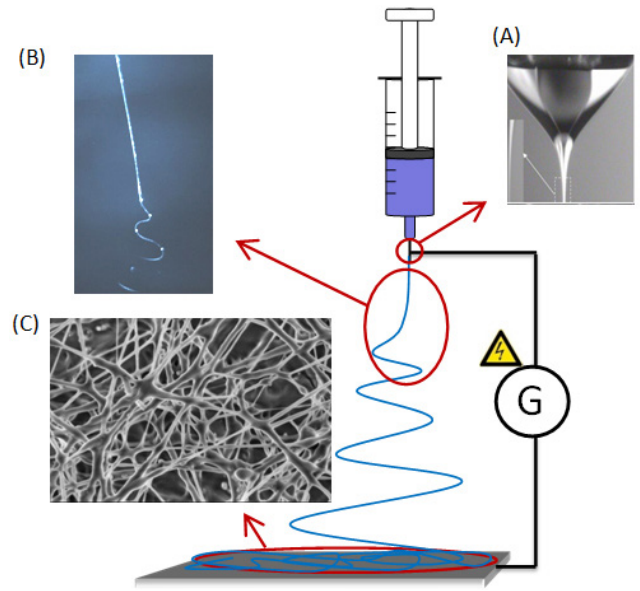

Figure 1: Principle of electrospinning.

Regarding the polymer solution, the molecular weight and the concentration that should be above the entanglement concentration in order to have fibers, the elasticity that should be high enough to avoid filament breakage, the conductivity of the polymer solution that favor filament stretching, the solution temperature and the vapor pressure of solution that control the solvent evaporation kinetics.

As mentioned above, Figure 2 illustrates the influence of polymer concentration.

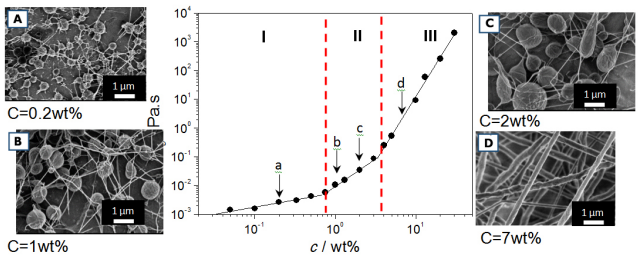

Figure 2: Newtonian viscosity of PEO solutions as a function of polymer concentration and morphologies obtained by electospun polymer solutions at **(A)** 0.2 wt%, **(B)** 1 wt%, **(C)** 2 wt%, and **(D)** 7 wt%. MW = 10^6 g/mol dissolved in water.

By increasing polymer concentration, we can obtain nanometric spheres to micrometric spheres in the dilute regime I, elongated shares in the semi-dilute regime II and finally fibers in the concentrated regime. In the last regime, fiber diameters increases with increasing polymer concentration.

The advantages of using the electrospinning process are the large diversity of available polymers, the high surface/volume ratio (10^3 times higher than micrometric fibers), and the high porosity (from 70% to 90%) with open pores, the large diversity in the structure formed and the biomimetic character of electrospun polymers.

The electrospinning process was performed with a horizontal setup – a 5 ml syringe was filled with polymer solution slightly above the overlap concentration and placed on the syringe pump with the blunt 21-gauge needle attached. Flow rate was controlled by a syringe pump (KD Scientific series 200, USA) in the range from 0.01 to 0.03 ml/min. Fibers were collected directly on aluminum foil. The distance between needle tip and collector was fixed at 15 cm. Applied voltage (dual high voltage power supply, ± 30 kV, iseg GMBH, Germany) ranged from 11 to 15 kV. All experiments were done at room temperature. The relative humidity noted was between 30 and 55%.

In the following, we discuss specifically electrospinning application for producing pure chitosan nanofibers mat to be used for cell development.

Chitosan characteristics

Chitosan was selected for this work, taking into account the original properties of this pseudo natural polysaccharide obtained from partial deacetylation of chitin [1]. Chitin is a component of crustaceous shells in addition of calcium salts and proteins. The molecular structure was given in Figure 3.

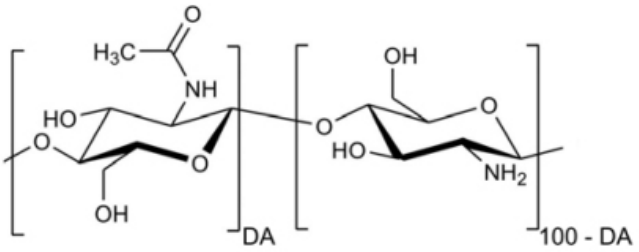

Figure 3: Schematic representation of chitosan based on D-glucosamine and N-acetyl-D-glucosamine.

The specific properties of chitosan are interesting especially for biological applications. As recognized, chitosan is a renewable polymer (chitin is the second polymer after cellulose, produced by different organisms from which exoskeleton of arthropods or cell walls of fungi and yeast). In addition, it is biocompatible, non-toxic, bioresorbable in the body, and hydrolysed by lysozyme. It is also a hydrophilic polymer with antifungal and antibacterial characteristics [2].

As soon as the degree of acetylation is lower than 0.5, it becomes soluble in acidic aqueous solutions at pH < 6.5. From those solutions, it may be easily processed under different morphology: beads, capsules, fibers, films, sponge or nanoparticles after pH increase over pH = 7.5 where chitosan is insoluble. For many applications, the exact chemical structure of chitosan is important and it controls the physicochemical properties: it depends on the average molar mass (MW), on the average degree of acetylation but also on the distribution of the acetyl groups along the chain (blockwise or random acetyl distribution).

Concerning the solid state of chitosan obtained after regeneration under slightly alkaline conditions, it is known as forming interchain H-bond network inducing high

mechanical properties for fibers and films.

Taking into consideration the advantages of chitosan, stable nanofibers made of pure chitosan were produced by electrospinning.

Applications

Since the early 2000s, major efforts are being made to develop new scaffolds dedicated to cell growth for tissue regeneration. Despite significant progress in this field, very few scaffolds for the reconstruction of soft tissues such as striated muscle, fibrous tissue, blood vessels or peripheral nervous system are developed and the reconstruction of these tissues do not receive any commercial solution up to now. In this context, only the reconstruction of tendons, ligaments and muscles and supporting tissues is now possible through the use of woven mesh mainly composed of polyester, poly (ethylene terephthalate) or polypropylene. However, these materials are generally not bioresorbable, and may cause long-term inflammatory phenomena responsible for the graft rejection that require to remove the implant. In some cases, for tendons and ligaments in particular, the implants do not allow the cell recolonization.

An alternative to the use of commercial implants is therefore to develop a new generation of implants for soft tissue combining both a biodegradable and biomimetic character, allowing its colonization by the host tissue while avoiding surgical removal action of the implant.

A major constraint on the development of scaffolds for soft tissue is clearly identified: to give these supports simultaneously a bioresorbable character, a biomimetic structure, and a mechanical behavior close to that of the tissue to regenerate. This difficulty, obvious at the early stage of the implant operation, is accentuated during the degradation of the bioabsorbable material.

For such nano-fibrous mats dedicated to tissue engineering, its structuration plays a key role to optimize the implants. The development of efficient biomimetic implants requires the development of three-dimensional structures having interconnected pores. Such structure must ensure the preservation of the mechanical properties but also allows the flow of biological fluids and therefore the transport of nutrients and cells that are essential for the process of cell growth and healing. To meet these requirements, the pore size and porosity must be controlled. Ideally, a pore size between 50 and 150 µm (or 5 to 10 times that of the cells) and a porosity greater than 80% promote adhesion and cell migration [3]. Thus, recent studies have attempt mainly to combine the biocompatible character of the support with a biomimetic cell morphology suitable for colonization by the host body [4]. Lyophilization of chitosan gel [5], mixtures of collagen/chitosan [6] or the phase separation of PLLA [7] were used to obtain micro-tubular porous structures with high potential for tissue engineering. 3D fast prototyping printer produces also centimeter scale porous scaffolds made of agar or alginate/fibrin mixtures for the reconstruction of nasal tissues [8]. If these techniques have undoubtedly generated great interest, they face difficulties in obtaining a sufficiently interconnected porosity and a controlled morphology of the structures produced. These difficulties significantly hinder the biomimetic properties of the obtained implants.

Drawing polymer nanofibers under intense electric field, known as electrospinning, was clearly identified as the most appropriate method for obtaining a fiber structure with very strong potential for tissue engineering [9-12]. Thus, anisotropic structures of bio-fibers controlled by the alignment of fibers have been proposed for the culture of fibroblasts [13], and electrospun structures have demonstrated their bioactivity [14] that favors the growth of keratinocytes and fibroblasts [15].

Chitosan is used in many biological applications such as surgical suture, dental implant, artificial skin, rebuilding bone, corneal contact lenses, time release drugs for animals, and encapsulating material. In this domain of applications, the chitosan based biomaterials are used for their immunologic, antitumoral, hemostatic and anticoagulant, healing, and bacteriostatic properties. Our objective is to produce nanofibers for tissue engineering. Due to large specific area, nanofibrous structure promoted the attachment of human osteoblasts and chondrocytes and maintained characteristic cell morphology [16].

As chitosan cannot be electrospun directly, blends with compatible polyethylene oxide (PEO) was used to favour spinnability. The main conditions were: chitosan/PEO weight ratio equal to 80/20, chitosan MW around 150 000, PEO MW around 1 x 10^6 and common solvent 0.5 M acetic acid using 5% (w/v) polymer solutions. After electrospinning, nanofibers under protonated form of chitosan were made of chitosan/PEO blends. Pure chitosan nanofibers were recovered by immersion in ethanol/water 70/30 (v/v) containing potassium carbonate (0.2 M) and washing in water [17, 18]. An example of the nanofibers obtained was given in Figure 4.

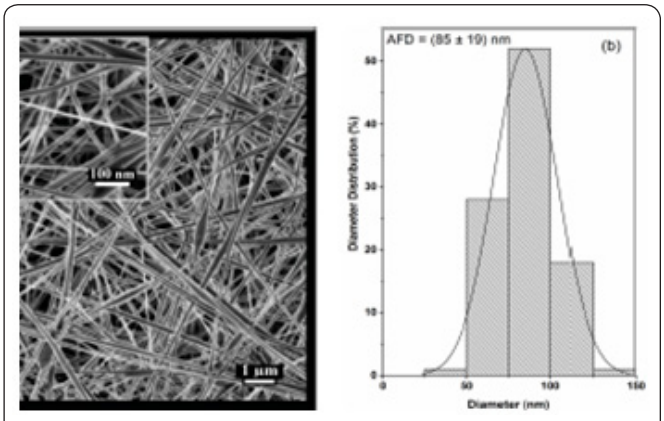

Figure 4: Representative SEM images and fiber diameter distributions of the electrospun nanofibers obtained from blend of powdered PEO into chitosan solutions (CS/PEO) at weight ratio 80/20. The insets show the high magnification images. The fiber diameter distributions of each are shown on the right. AFD: average fiber diameter equals 85 nm.

The mechanical properties of these nanofibers were determined on the mats under the dried and the wet states. This highly porous material was hydrophilic (with a water retention around 4 g water per g of dried fibers) with around 40% extension degree. The density of the nanofibers mat was around 0.1 depending slightly on the experimental conditions adopted.

As shown in Figure 5, applications of the electrospinning technique in health is the major application in terms of publication and patents.

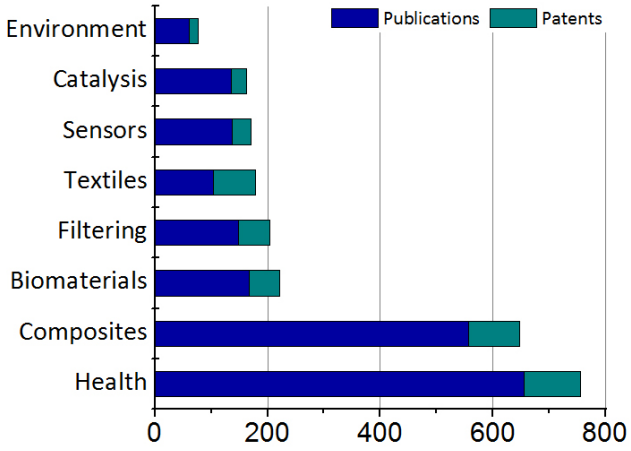

Figure 5: Breakdown by areas of application for patents and publications on the electrospinning since January 2014.

However, the use of electrospun fibers is promising in composite applications as enhancement agent [19], in the development of sensor and new electronic devices [20, 21], in catalysis due to the high surface/volume ratio [22], as new material to produce ceramic [23, 24]. A global description of the application and new perspectives are depicted in the recent review of C J. Xue et al. [25].

Conclusion

Electrospinning technique was adopted to produce nanofibers made of pure chitosan proposed for cell development. The original properties and advantages of chitosan have been described as well as the importance of parameters involved in electrospinning. Our purpose is to extend this work to prepare nanofibers using modified chitosan or blends with other biocompatible hydrophilic natural polymers to optimize the cell adherence on the mats.

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