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REVIEW

Chitosan films for regenerative medicine: fabrication methods and mechanical characterization of nanostructured chitosan films

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Abstract

Regenerative medicine is continuously facing new challenges and it is searching for new biocompatible, green/natural polymer materials, possibly biodegradable and non-immunogenic. Moreover, the critical importance of the nano/microstructuring of surfaces is overall accepted for their full biocompatibility and in vitro/in vivo performances. Chitosan is emerging as a promising biopolymer for tissue engineering and its application can be further improved by exploiting its nano/microstructuration. Here, we report the state of the art of chitosan films and scaffolds nano/micro-structuration. We show that it is possible to obtain, by solvent casting, chitosan thin films with good mechanical properties and to structure them at the microscale and even nanoscale level, with resolutions down to 100 nm.

Keywords Chitosan · Micrograting · Nanostructured surfaces · Solvent casting · Nerve regeneration

Introduction

Tissue engineering is an interdisciplinary field that aims to use an implantation (i.e., scaffold) as (temporary) support to repair, replace, or enhance the function of a particular tissue. The ultimate aim is to develop a scaffold that can interact with the living tissue and stimulate and support its spontaneous regeneration (Shafiee and Atala 2016). Scaffolds can be optimized in chemistry, geometry, and functionalization (i.e., with biological factors, such as growth factors, or drugs) in order to better interact with patient's cells (Lee et al. 2011; Almeida and Bártolo 2013). The scaffold acts as a temporary extracellular matrix (ECM), thus guiding cell behavior and tissue progression, until it is completely regrown (Ma 2004).

Typically, a scaffold is described as a three-dimensional solid support made of biomaterials. An ideal biomaterial is commonly defined as biocompatible, biodegradable, non-cytotoxic/non-mutagenic with respect to its degradation products. Accordingly, a biomaterial can promote cellular interaction, cell adhesion, and extracellular membrane deposition, all necessary steps to improve the subsequent cell proliferation on the surface and the final tissue regeneration (Ghassemi et al. 2018; Zhang et al. 2018). A key feature of any scaffold is its mechanical stability: it should physically sustain the tissue regrowth before biodegradation occurs (Bitar and Zakhem 2014).

Consequently, new materials, suitable for tissue engineering, are the object of continuous scientific research (Chan and Mooney 2008). Recent trends have placed the focus on natural biomaterials that do not have a high footprint on the environment and, at the same time, are not expensive and easy to be molded (Jahangirian et al. 2018). Biopolymers are a wide category of materials whose main sources are living organisms, not only plants and animals but also microorganisms (Rao et al. 2014).

One of the main reasons behind the choice of nature-derived materials employed as scaffolds is their high biodegradability. In addition, their biological origin often makes them favorable to interact with the biological systems (Le Bao Ha et al. 2013). Natural polymers can be further classified for their chemical composition: polysaccharides (cellulose,

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starch, chitin, and glycosaminoglycans) or proteins (keratin, collagen, silk, elastin, and fibrin); polynucleotides are less used in this field (Ratner et al. 2004). Natural polymers, such as collagen or gelatin, are the first biodegradable materials employed in human clinical practice (Nair and Laurencin 2007). Compared with synthetic materials, they tend to perform a greater biological interaction with cells and to have fewer side effects, such as toxic biodegradation products (Barua et al. 2018). For instance, collagen has a good biological interaction with cells: being the main fibrous structural protein in our body, it is non-immunogenic and resembles the cell's native environment (Dong and Lv 2016). However, its mechanical properties and fast biodegradability are strong limitations (Ma et al. 2003).

Chitosan as a promising material for regenerative medicine

Chitin is one of the most abundant polymers present in nature, second only to cellulose (Elieh-Ali-Komi and Hamblin 2016). It is a natural homopolymer of N-acetyl-D-glucosamine widely found in the exoskeletons of arthropods and insects and in crustacean shells as well as in fungi cell wall. From the controlled deacetylation of chitin, it is possible to obtain a copolymer of β(1-4)-linked N-acetyl-D-glucosamine and D-glucosamine subunits, called chitosan (Islam et al. 2017). In nature, the extracted chitin is usually bound to proteins and minerals, which can be removed through processes of acidification and alkalization (Tapan Kumar and Bijaya 2018). The purified chitin is then converted into chitosan through controlled chemical processes, with the tuning of parameters such as concentration, ratio of chitin to alkali and temperature, in order to obtain a precise deacetylation degree in the final product (Sorlier et al. 2001). The degree of deacetylation impacts on the biological properties of chitosan, such as cell adhesion, healing capacity, and breakdown processes. Another important parameter is the molecular weight that depends on chitosan preparation procedures. It correlates with viscosity and it is inversely proportional to swelling capacity (Rodríguez-Vázquez et al. 2015).

The use of chitosan as a biomaterial is approved by the Food and Drug Administration (FDA) for application in biomedical devices, in particular, in drug delivery and in tissue engineering, with the final goal to restore the functionality of defective or lost tissues. As already hinted, chitosan is a completely biodegradable material; through an enzymatic transformation, it is broken down to its basic, non-toxic building blocks. In vivo, there are several enzymes that promote its degradation: the predominant one is lysozyme, a non-specific protease found in all mammalian tissues (Szymańska and Winnicka 2015). Importantly, chitosan is a hypoallergenic and bio-tolerated material: it does not routinely stimulate inflammation when implanted (Rodríguez-Vázquez et al. 2015). Moreover, it has shown interesting antimicrobial and antifungal properties and, for this reason, it is intensely studied for food packaging (Fernandez-Saiz 2011; Gutiérrez 2017) and tissue engineering applications (Rodríguez-Vázquez et al. 2015). The physicochemical properties of scaffolds based on chitosan depend mainly on two parameters: the degree of deacetylation and the molecular weight of the starting material. For biomedical applications, a high degree of deacetylation is preferred, because this parameter has also an impact on the biological properties of the biomaterial, such as the degradation time in vitro and in vivo (Wei Wang et al. 2006).

Thanks to its biodegradability, chitosan has been extensively employed in medicine not only as scaffold material but also as a material of choice for the synthesis of nanoparticles for non-parenteral drug delivery of many drugs and vaccines, via several routes of administration (Mohammed et al. 2017). In particular, the ability to open the tight junctions in the epithelia makes it ideal for mucosal delivery, increasing the paracellular permeation and, as a consequence, the adsorption of the nanoparticles (Sonaje et al. 2012). This polymer can be also variously modified to finely tune the degradation pH and time and so modify the pharmacokinetic profile of drug release (Yuan et al. 2013; Miladi et al. 2015; Fonseca-Santos and Chorilli 2017).

Since the main topic of this review is the use of chitosan for regenerative medicine, we will focus on the chitosan employment for the fabrication of scaffolds for tissue engineering. In fact, it was already shown in literature that addressed tissue can be various (skin, bone and cartilage, nerve tissue, liver, heart, or cornea), as in the case of applications (Dutta, Rinki, and Dutta et al. 2011).

Chitosan has been used alone or in combination with other materials, in order to enhance the mechanical properties and degradation time for scaffolds. For instance, employment of chitosan would not be useful for skin tissue repair, but it can be an effective modifier for scaffolds made of polymers that, like collagen, have limitations in terms of rapid biodegradation and poor mechanical properties (Romanova et al. 2015). In order to avoid the short-time degradability and to enhance its mechanical properties, collagen was combined with chitosan, enhancing the scaffold stability over time (Tangsadthakun et al. 2006).

Bone tissue engineering aims for the construction of scaffolds that are mechanically strong enough to sustain bone regrowth. Usually, scaffolds for bone tissue are made of combinations of polymers and ceramic materials, such as calcium phosphate (Saravanan et al. 2016). Chitosan, thanks to its biodegradability and biocompatibility, is a good candidate for this medical application. Chitosan has been mixed with hydroxyapatite to create an ideal matrix for osteoblast proliferation and mineral deposition (Zo et al. 2012). Another possibility is to complex chitosan with whitlockite (an unusual form of calcium phosphate). Comparing the whitlockite/chitosan with the hydroxyapatite/chitosan composites, the first

composite material shows better biocompatibility and enhances osteoblast proliferation (Zhou et al. 2017).

Peripheral nerve regeneration is one of the research fields in which chitosan, even when employed alone, shows the best results in terms of regeneration performances. One of the main options for the repair of short (below 3 cm) nerve gaps is becoming the implantation of a nerve guidance conduit, a tubular scaffold that connects the two ends of the injured nerve and sustains the regeneration process (Lundborg 2000; Ijpma et al. 2008; Sachanandani et al. 2014; Subramanian et al. 2009; Zeugolis et al. 2011). Chitosan-based nerve conduits, alone or in combination with other biomaterials, have been found to bridge efficiently peripheral nerve defects (Gnavi et al. 2013). Apart from rats (Gonzalez-Perez et al. 2015; Fregnan et al. 2016), chitosan conduits have been tested in several animal models for nerve regeneration, such as dogs (Tanaka et al. 2015) and goats (Muheremu et al. 2017). To provide an example, chitosan nerve conduits having an internal longitudinal chitosan membrane were used on a 10-mm sciatic nerve defects in adult healthy and diabetic rats and provide an enhancement in functional and morphological nerve regeneration (Meyer et al. 2016). In another work, chitosan flat membranes, crosslinked with dibasic sodium phosphate, were fabricated with a solvent casting technique (Fregnan et al. 2016). In vitro, the membranes allowed Schwann and DRGs' cell proliferation and in vivo promoted nerve functional recovery, but leading only to an outcome comparable to median nerve repaired by autograft. Again, for the repair of long-gap peripheral nerve injury in the rat, the results with chitosan tubes (with varying degree of acetylation) were significantly better compared with silicon tubes, but lower than those with autografting (Gonzalez-Perez et al. 2015).

In fact, despite recent developments in biomaterial-based artificial scaffolds (Daly et al. 2012), autografting (with the related donor-site morbidity) still remains the gold standard in the clinical practice for nerve reconstruction (Raimondo et al. 2011), in particular for large nerve gaps. Chitosan has been already approved for clinical use in Europe. Reaxon® Nerve Guide conduits are smooth chitosan conduits, sold with different diameters (from 2.1 to 6 mm) and 3 cm long, to bridge gaps up to 26 mm. They are promoted as biocompatible, antibacterial, and antiadhesive, limiting scar tissue formation (Neubrech et al. 2016). When compared with autologous nerve grafts, the classical gold standard treatment for nerve injuries, Reaxon conduits gave similar results, with no statistically significant difference in the healing process (Shapira et al. 2016). The use of nerve guidance conduits for small diameter nerves has shown promising results, with most of the human studies describing neuronal recoveries between 74 and 100% (Braga Silva et al. 2017). However, there are still problems in repairing large-diameter nerves and wider gaps (Rebowe et al. 2018) and enhancing the regenerative potential of conduits could help in facing these limitations.

Chitosan topography modifications

Nowadays, chitosan films and conduits have been mainly modified in their chemical composition, by adding other materials (e.g., synthetic polymers, nanofillers) or cells (Gnavi et al. 2013), with less efforts in tuning their physical features.

Cells in vivo are embedded in a complex textured environment, composed of ECM meshed nano/microfibers (Tuzlakoglu et al. 2005; Wade and Burdick 2012; Andalib et al. 2016). It is a 3D physical environment composed by factors secreted by cells, mainly proteoglycans and fibrous proteins (Frantz et al. 2010). The ECM conveys not only biochemical but also physical cues to cells, triggering then an intracellular signaling cascade: this phenomenon is called mechanotransduction (Shih et al. 2011; Steward and Kelly 2015; Smith et al. 2017; Wolfenson et al. 2018). Hence, cells can respond to topography, at microscale and even nanoscale levels.

It was recently demonstrated that, by changing the surface topography at the nano/microscale, it is possible to control and guide the behavior and differentiation of a cell to a particular phenotype, changing its fate (Ferrari et al. 2010a, 2010b, Ferrari et al. 2011; Ankam et al. 2013; Franco et al. 2013). Not only the differentiation but also other processes involved in tissue regeneration can be regulated by substrate topography, such as cell polarization, neurite growth, and migration. Cell migration can be tuned by nano-microstructured surfaces and in particular significantly directed/enhanced with anisotropic topographies, such as nano/microgratings (Cecchini et al. 2008; Ferrari et al. 2010b; Jacchetti et al. 2014; Tonazzini et al. 2014a). These topographies (i.e., alternating lines of ridges and grooves with (sub) micrometric dimensions) have been designed and optimized in dimensions accordingly to the cell type and application (Tonazzini et al. 2014b), to promote neurite growth, cell polarization, and cell migration in the desired direction. Human endothelial cell migration is enhanced on gratings with a 2-μm period (Antonini et al. 2015). It has been shown that primary rat Schwann cells migrate faster on gratings with a 20-μm period (period = ridge width + groove width), analyzed as single cells, while their collective migration (i.e., in a monolayer, simulating a tissue wound healing situation) is enhanced on gratings with a 4-μm period (Tonazzini et al. 2015).

As already stated, chitosan is one of the major candidates as a suitable material for regeneration applications (reviewed in Rodríguez-Vázquez et al. 2015). Overall, chitosan, although an environmentally friendly promising biopolymer itself for regenerative medicine (Jahangirian et al. 2018), could be further improved in its regeneration potential by the introduction of topographical cues for cells, in order to direct their migration/differentiation and speed up the healing process.

There are a few fabrication techniques that have already been used to create nano/microstructures on chitosan films

or scaffolds, but not all of them can be used for a precise and directional nano/micro-topography structuration. (i) Electrospinning is a useful manufacturing technique to obtain micro/nanofibers and mimic the texture of the ECM (e.g., collagen fibers). In fact, nanofibers have been effective in improving Schwann cells' healing (Tonazzini et al. 2017). Wang and coworkers successfully created a chitosan nano/microfiber mesh tube (Wang et al. 2006). The chitosan solution (5%, in trifluoroacetic acid and methylene chloride) was electrospun on a negatively charged steel use stainless bar, which was intermittently compressed during chitosan deposition, compacting the fibers into a tube. Depending on the degree of chitosan deacetylation (DAc), the obtained fibers were 200 nm (for DAc 93%) or 400–600 nm (for DAc 78%) in diameter, with 10.98 MPa and 5.30 MPa Young's moduli respectively. The chitosan tube made of 200-nm fiber mesh (DAc 93%) performed as the best conduit for nerve regeneration; however, its regenerative outcome was lower or comparable than for the iso-grafting control group. Additionally, 3D nanofibrillar chitosan scaffolds have been developed by electrospinning for skin regeneration and demonstrated to induce a faster regeneration of both the epidermis and dermis compartments, both in vitro and in vivo (while 3D chitosan sponges developed by freeze-drying induced granuloma formation) (Tchemtchoua et al. 2011). (ii) Nanosphere lithography (NSL) was used to generate surfaces of chitosan that mimic the nanostructures found on the surface of certain insect wings (Chandran et al. 2018). In NLS, the substrate is firstly covered with a close packed nanosphere layer and then processed by material deposition or etching. Here, chitosan, such as chitosan/nanosilver particles, was also able to self-assemble in a self-masking thin film, thus enabling a novel tool for the NLS. Both with classic NLS and the self-masking techniques, it was possible to obtain nano-cone patterns of about 250 nm in diameter. Regrettably, this is the only type of topography that is possible to obtain with these techniques and was not tested further in vitro or in vivo. (iii) Freeze-drying has been used to create three-dimensional chitosan-based–structured scaffolds. Yin and coworkers built a chitosan nerve conduit with highly aligned, double-layered porosity, having an overall control on pore size and orientation through the materials used to shape the scaffold (Yin et al. 2018). The solution (3.5 w/v% chitosan in 1.5 v/v% acetic acid) was injected into a coaxial tube, having the external part in aluminum and the internal one in brass. The system was frozen and subsequently lyophilized. This resulted in the formation of two distinct structural chitosan layers, with differently oriented and shaped pores, due to the two opposite thermal gradients during freezing generated by the two materials. The conduits were tested for compression showed a fully recoverable behavior, with an initial low stiffness for compression and a higher resistance due to compaction of the wall. These conduits were evaluated in bridging 10-mm Lewis rat sciatic nerve gap at 12 weeks post-implantation and qualitatively showed good regenerative efficacy.

Even though there are several reports about 2D and 3D chitosan biomaterials, chitosan has been mainly exploited for nanoparticles and nanocarriers and chitosan-based films or scaffolds have been mainly fabricated with no surface structuration, into tubular forms or at most into nanofiber scaffolds (Elieh-Ali-Komi and Hamblin 2016). There are very few studies about the precise micro/nanopatterning of chitosan films. The above-mentioned techniques cannot transfer a defined geometrical pattern on a chitosan membrane with micrometer or nanometric dimensionality. At the moment, chitosan has been patterned using only two techniques: (1) low-pressure low-temperature nanoimprinting and (2) solvent casting.

1. Nanoimprinting is a process that enables the imprinting of a pattern onto a thin film of a second (usually thermoplastic) material (Truskett and Watts 2006). The film is pressed on a silicon or polymeric mold with the desired pattern, with controlled high pressure and temperature. After the removal of the mold, the pattern is reproduced, inversely, on the film. A modified version of the nanoimprinting technique was used to produce chitosan films: it is slightly different from the classical nanoimprint process (carried at high temperatures), since it takes advantage of the relatively low temperature solidification of chitosan (Park et al. 2007). Medium molecular weight chitosan was dissolved in a solution of acetic acid 50% and heated at 40 °C to form a hydrogel, without using any additional plasticizer to lower the viscosity of the chitosan solution. Polydimethylsiloxane (PDMS) molds with micrometric and nanometric patterns were created as follows: microstructures consisted of microwells (3.5 μm diameter and 220 nm depth), microposts (2.2 μm width and 350 nm height), and checkerboards (2.2 μm width and 280 nm height); nanostructures consisted of nanowires (150 nm width and 500 nm pitch) and nanodots (150 nm width and 400 nm pitch). Molds were pressed on drops of chitosan solution, applying moderate heating (90 °C) and pressure (5–25 psi). Overall, the smallest features replicated had the resolution of 150 nm in width. This method successfully conveyed nano- and microfeatures on chitosan films, for bionanodevice applications. However, this method required several passages, careful control of the viscosity, and the use of a nanoimprinter and therefore of clean room facilities.
2. Solvent casting is probably the easiest method to fabricate plain or structured chitosan substrates. With this technique, a polymer solution is poured on a patterned mold, previously created with lithography techniques (Siemann 2005). Then, the solvent is allowed to evaporate, leaving a solid chitosan film that can be peeled off the mold. This method has been recently used to structure chitosan from

silicon molds, creating micropatterned substrates (Sung et al. 2015). A 1% chitosan solution was poured on micropatterned silicon molds created by photolithography and left overnight at 60 °C. The molds had squared (50 μm wide and 5 or 15 μm deep), line, and hexagonal-like geometric patterns, with flat area surfaces printed on nanotextured regions created by Ag nanoparticle-assisted etching. Here, the solvent casting technique achieved good results in replicating both the micrometric features and nanotexturization. Neuro-2a cells preferred to adhere to the flat chitosan surfaces rather than the nanotextured areas and the hexagonal-like micropattern provided the most suitable surface for promoting neural cell network formation on these chitosan substrates in vitro. Though promising, Sung's work is the only example of chitosan micropatterning with this technique, at the best of our knowledge.

Towards the nanoscale: fabrication of nano/micropatterns on chitosan membranes

Bio-based chitosan biopolymer scaffolds reinforced with nanostructures are emerging therefore as an interesting, but not yet deeply investigated, area of research. The easiest technique to obtain thin chitosan films is solvent casting. This technique does not involve complex instrumentations, high temperature, or harsh chemicals; it preserves the biocompatibility of the material and avoids its thermal degradation. We therefore set up a protocol to develop micro- and nanostructured chitosan thin films with topographical patterns of gratings (GRs; i.e., alternating lines of ridge and grooves) that can induce directional stimuli to cells (Tonazzini et al. 2015). The aim was to assess to which extent solvent casting was able to replicate nanoscopic features on chitosan films.

Chitosan was purchased in three different molecular weights, classified as low, medium, or high (Sigma-Aldrich). A 2% w/v chitosan solution (in distilled water + acetic acid 1% v/v) was filtered with a filter paper having a 10-μm cut-off (Superfiltro, Milano, IT) and poured on different prefabricated (methods in Masciullo et al. 2018, Masciullo et al. 2017) cyclic olefin copolymer molds having GR patterns of decreasing period: (1) a mold having 4 μm of period and 370 nm of depth (T4); (2) a mold with GRs with a 400-nm period and 200 nm of depth (T400); (3) a mold with GRs of a 200-nm period and 90 nm of depth (T200). Chitosan solution was baked at 37 °C until complete evaporation of the solvent. After evaporation, thin (~ 300 μm) chitosan films were peeled off of the molds (Fig. 1a) and evaluated by scanning electron microscopy (SEM) with a LEO 1525 field-emission scanning electron microscope (Zeiss).

As demonstrated by the SEM images reported in Fig. 1b, all of the three different molecular weights (low, medium, and high) of chitosan were able to replicate the GR features, from T4 down to T200 with no differences in the replication effectiveness. It was possible to distinguish well-defined ridges and grooves, with the expected period imposed by the mold. Even the smallest GR pattern (T200) was finely replicated on the chitosan surfaces. Altogether, this solvent casting technique allowed us to replicate nanostructured directional features on chitosan films with an overall resolution down to 100 nm, for the first time. This result is an important achievement: it demonstrates that it is possible to obtain chitosan films with the desired nano-GR pattern with a solvent casting technique that does not impact on the biological and mechanical properties of the material; it is simple (e.g., no clean room facilities need for the process itself, while any original mold can also be easily purchased on the market) and cost-effective (e.g., high mold reuse). This protocol represents an easy process to create nanostructured scaffolds for tissue engineering.

Mechanical characterization of chitosan membranes

The regeneration process is also influenced by the mechanical properties of the scaffold. An artificial substrate conveys to cells' physical signals (e.g., stiffness) that regulate many processes in regeneration, such as cell proliferation and migration. For this reason, the mechanical compatibility of the material is fundamental in determining the outcome of the regeneration process and the scaffold would rather resemble the mechanical properties of the native tissue.

Chitosan films were mechanically characterized by uniaxial tensile tests at a constant cross-head speed using an Instron 5564 Testing System (Instron, Norwood, USA) equipped with a 2-kN load cell (Puppi et al. 2016). These measurements were performed again on the three different molecular weights of chitosan (low, medium, and high). Thin films of chitosan were prepared by solvent casting on silanized silicon wafers. Chitosan scaffolds are brittle when dry (such as before implantation), while their mechanical properties change when soaked in a liquid (such as in body fluids). For this reason, the mechanical properties of both dry and wet films were measured, in order to determine the different behavior of chitosan films in the two conditions. For the preparation of wet samples, dry chitosan films were first neutralized with NaOH 1% w/v for 30 min and then rinsed with deionized water.

Dog-bone–shaped (21.1 × 4.75 × 0.90 mm) samples were tested at a strain rate of 10 mm/min until specimen failure. The test was conducted at room temperature on 7 replicates for each molecular weight and dry/wet test condition. By analyzing the obtained stress-strain curves, the Young's modulus (MPa) was calculated as the slope of the initial linear region, while the stress

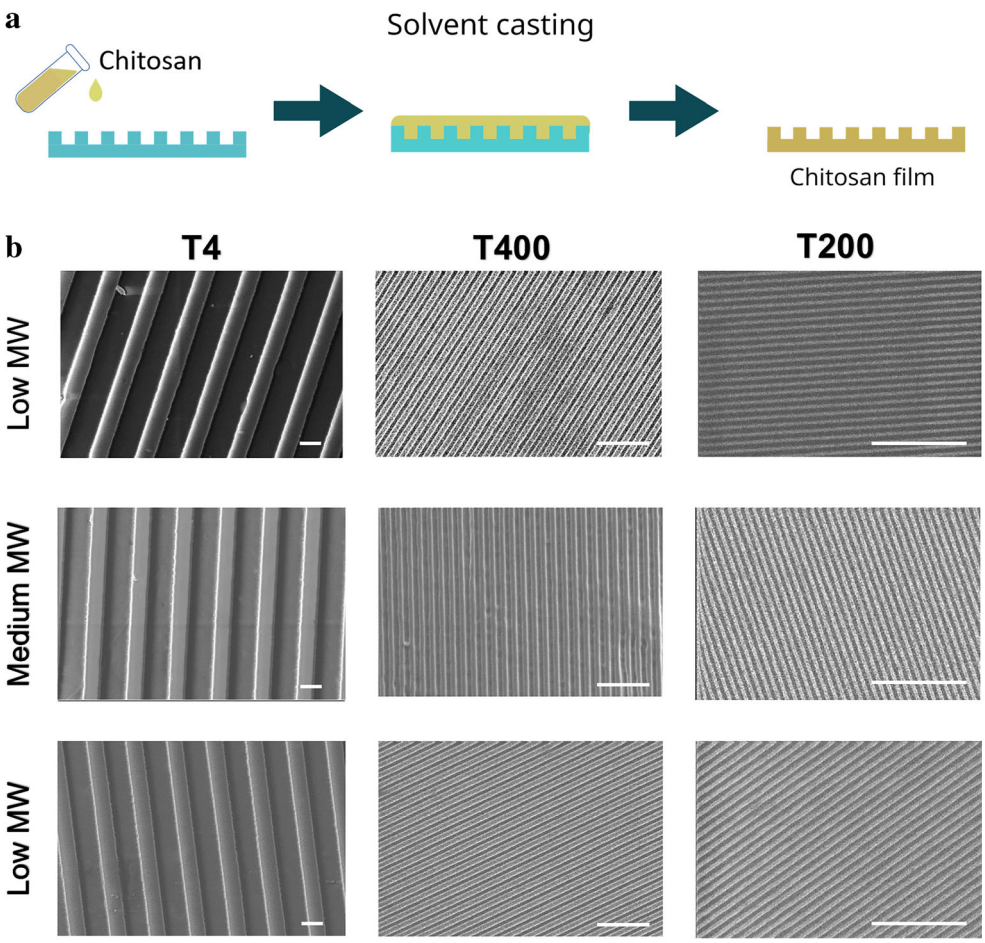

Fig. 1 **a** Schematic representation of the chitosan patterning process. **b** SEM images of microstructured and nanostructured films made of chitosan with low, medium, and high molecular weights; scale bars = 2 μm

(MPa) and strain (%) at break were obtained at the sample break point. The measured values are reported in Table 1.

As expected, the Young's modulus and stress at break of wet films were consistently lower than those of dry films, for all molecular weights. On the other hand, the strain at break was significantly larger when samples were tested in wet conditions. The explanation for this behavior is that water acted as a plasticizer and enhanced the elasticity of the material.

For optimal tissue regeneration, there should be a match between the mechanical properties of the native tissue and the ones of the material. The stiffness of the material should be as close as possible to the natural environment of the cells we are trying to regenerate. For instance, the nerve Young's modulus is about 0.58 MPa (Borschel et al. 2003), roughly an order of magnitude lower than medium Mw chitosan films (5 MPa) and only one-third that of high Mw chitosan films (1.8 MPa) tested in wet conditions.

Several factors other than material mechanical properties need to be considered, first of all the biocompatibility, but also the swelling due to degradation/fluid absorption, and the implantation procedure/suturability (if needed). An effective compromise between all these parameters should be optimized.

Table 1 Mechanical properties of low, medium, and high molecular weights (MWs) chitosan films in dry and wet conditions. All values are reported as mean ± standard deviation

	Young's modulus (MPa)	Stress at break (Mpa)	Strain at break (%)
Low Mw-dry	1644 ± 400	35 ± 9	18 ± 5
Medium MW-dry	2782 ± 500	56 ± 10	19 ± 4
High Mw-dry	430 ± 60	8.6 ± 1.5	15 ± 7
Low Mw-wet	2.9 ± 0.5	3.9 ± 1.5	123 ± 35
Medium Mw-wet	5 ± 1	5 ± 1	130 ± 11
High MW-wet	1.8 ± 0.4	1.6 ± 0.3	108 ± 13

Conclusions

Regenerative medicine is facing new challenges and it is continuously searching for biocompatible and green/natural polymeric materials that are possibly biodegradable, non-immunogenic and having a good interaction with biological systems. Moreover, it is overall accepted that the nano/microstructure of (chitosan) devices deeply affects their regenerative performances. Chitosan is emerging as a promising biopolymer for tissue engineering and its application can be further improved by exploiting its nano/microstructuration, creating topographical features in chitosan membranes and conduits. Here, we reported the state of the art of chitosan films and scaffolds nano/micro-structuration and showed that it is possible to structure chitosan films at the microscale and even nanoscale levels, with resolutions down to 100 nm. Chitosan mechanical properties have been also characterized, as preliminary information on the possible use of this material in tissue regeneration. Further studies will confirm the enhanced regenerative potential of micro/nanostructured chitosan as scaffolds for nerve regeneration.

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