Alginate-based fibers loaded with ciprofloxacin hydrochloride

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Abstract

Introduction

Electrospinning is a simple and versatile method for fabrication of continuous and uniform polymeric nanofibers. Various synthetic polymers (*e.g.* PCL, PLA, PGLA, *etc.*) have been successfully electrospun into ultrafine fibers [1-3]. While natural polymers (*e.g.* chitosan, sodium alginate, hyaluronic acid, *etc.*) need to be usually electrospun in blend form by mixing them with synthetic polymers such as PEO, PVA or PVP, which improves their electrospinning processability [4-8]. High specific area, tunable pore size, controlled mechanical properties, and their ability to interact with cells in a manner which mimics the natural ECMs mainly cause that fibrous materials are finding an increasing range of applications, including biomedical areas *i.e.* antibacterial fibers for wound dressings, scaffolds for tissue engineering (*i.e.* skin, bone, cartilage and cardiac tissue), drug delivery systems, medical implants, protective textiles, filtration systems, *etc.* [8-12].

Electrospinning is affected by the following parameters and variables: *i*) polymer properties: molecular weight, molecular structure (branched, linear, *etc.*), *ii*) solution

properties: viscosity, conductivity, dielectric constant, and surface tension, *iii*) process parameters: electric potential, flow rate and solution concentration, distance between the capillary and collection screen, needle diameter; *iv*) ambient parameters: temperature, humidity and atmosphere pressure, and finally *v*) technical parameters: motion of the target screen [13, 14].

In general, electrospun nanofibers revealed great capacity for wound dressing due to their unique properties such as high surface area, swelling capability for efficient adsorption of excess exudates, adjusting the wound moisture, oxygen permeability for respiring, effective assistance in wound protection from exogenous microorganisms, and systematic drug administration in case of drug delivery systems based on fibrous mats [15]. Furthermore, antimicrobial properties of wound dressings play a key role in determining the process of wound healing because wounds often provide favorable environments for colonization of microorganisms, which may lead to infection and delay healing. In particular, when alginate is combined with well-known biocides (*e.g.* antibiotics, metal nanoparticles, *etc.*) it forms an effective antibacterial wound dressing, that offers many advantages including hemostatic capability, gel-forming ability upon absorption of wound exudates and last but not least well-defined drug delivery system. For instance, it has been suggested that alginate dressings (*e.g.* Kaltostat®) can enhance wound healing by stimulating monocytes to produce elevated levels of cytokines such as interleukin-6 and tumor necrosis factor-α. Production of these cytokines at wound sites results in pro-inflammatory factors that are advantageous to wound healing [16].

Alginate was found to possess many significant and desirable properties as material for wound dressing such as good water absorptivity, conformability, optimal water vapor transmission rate, and mild antiseptic properties coupled with non-toxicity, non-immunogenicity, and biocompability [4, 8, 17]. What is noteworthy, alginate is inherently non-degradable in physiological conditions, however when ionically cross-linked in form of a gel can dissolve *via* a loss of divalent ions into the surrounding media rather than real degradation. Fortunately, the molecular weights of commercially available alginates are typically above the renal clearance threshold of the kidney [8].

Electrospinning of alginate is still challenging task, since sodium alginate is a polyelectrolyte having high conductivity and surface tension. Even though alginate can form solutions with a wide range of viscosity, in this case viscosity is not a limiting factor but the repulsive force among the polyanions are the key factor hindering electrospinning of sodium

alginate [14]. These repulsive forces can be reduced by interactions between PEO and sodium alginate occurring after blending. Thus it allows successful electrospinning of sodium alginate/PEO blends [6, 7].

In this paper we present a simple method to increase and adjust the stability of sodium alginate electrospun nanofibers in aqueous solution by blending alginate with poly(ethylene oxide). Fibers were loaded with Ciprofloxacin, a fluoroquinolone antibiotic that is widely used for wound healing applications. In addition, we also show a method how to minimize the cytotoxicity involved in preparing the electrospun mats by application of only biocompatible materials. The use of biopolymers as well as non-toxic solvents and crosslinkers permits the fabrication of nanofibrous scaffolds for potential application in wound healing, regenerative medicine and as drug delivery systems.

Materials and methods

Materials

Sodium alginate (AL, alginate characterization, MW alginate), poly(ethylene oxide) (PEO, M_w 100, 600, 1000 and 2000 kDa), Triton X-100, Pluronic F-127, ciprofloxacin hydrochloride (CpHCl), ethanol and calcium chloride were purchased from Sigma-Aldrich and used without any further purification.

Fabrication of alginate-based electrospun fibers loaded with ciprofloxacin hydrochloride

Sodium alginate, PEO and ciprofloxacin hydrochloride were dissolved in water at the desired final concentrations in ranges from: 1-5 wt.%, 1.5-3 wt.% and 0.02-0.48 wt.% (0.2-4.8 mg/ml), respectively. Then, the surfactants: Triton X-100 or Pluronic F-127 were added at final concentrations of 0.5-1 wt.%. The prepared mixtures were stirred overnight at room temperature.

An Yflow 2.2 D500 electrospinner with a coaxial setup was used to obtain the fibers. that were collected on a plate covered with Parchment paper for easy removal of mats. The polymer solution was pumped through a syringe with a 22 gauge needle. The pump was working at flow rate of 0.1-1.0 ml/h. The distance between the end of the needle and the collector plate was fixed at 15-20 cm. A voltage was varied from 6 to 10 kV until a stable

Taylor cone was achieved. All nanofibers were obtained at room temperature and relative humidity 30-50%.

Crosslinking and stabilization of alginate-based electrospun fibers loaded with ciprofloxacin hydrochloride

After electrospinning fibers were mechanically removed from the collector plate and ionically crosslinked. Fibers were soaked in ethanol (1 min), followed by calcium chloride solution (2 wt.%) in 1:5 ethanol:water (10 min). Subsequently, fibers were removed from calcium chloride solution and lyophilized.

Characterization of the electrospun fibers

Fiber mats were characterized by a scanning electron microscope (SEM, FEI XL30). Fiber diameters and standard deviations on 200 fibers per sample were measured using ImageJ program.

Ciprofloxacin hydrochloride encapsulation efficiency

The ciprofloxacin concentration was determined by UV-VIS spectrophotometry at 277 nm (Lambda 35, Perkin Elmer). A standard calibration curve for ciprofloxacin were prepared. Loading efficacy was calculated by the following equation (1):

$$LE\% = \frac{T - F}{T} \times 100\% \quad (1)$$

where T and F are respectively total and free amount of ciprofloxacin. Total amount of drug was recognized as an initial concentration of CpHCl in the synthesis solution. The free CpHCl concentration was calculated according to the calibration curve equation.

Release of ciprofloxacin hydrochloride

The release process was carried out in a continuous mode using a Shimadzu LC-10AT VP syringe pump. Crosslinked fibers were put into the syringe and rinsed with appropriate buffers (PBS and acetate buffer) at 37ºC. Rinsed solution was collected in 1.5 ml eppendorf

vials in different time intervals (1-10min; 20-60min; 1h-6h). The flow rate was 1ml/h, time of the whole ciprofloxacin hydrochloride release was 6 h. Collected samples were analyzed by UV-VIS spectrophotometry at 277 nm (Lambda 35, Perkin Elmer). Amount of the released ciprofloxacin was determined from ciprofloxacin calibration curve.

Results and discussion

Effect of additional polymer on formation of electrospun alginate fibers

Due to the high viscosity and high electrical conductivity of alginate solutions, formation of fibrous structures by electrospinning of pure alginate is really difficult and leads to generation of sprayed droplets or short fibers embedded with beads. Another cause that limited the electrospinnability of aqueous sodium alginate solutions is the high surface tension. To solve these problems different approaches were used (*i*) incorporation of an additional well-electrospinable polymer, (*ii*) application of surfactants or/and (*iii*) addition of co-solvent to alginate solution [18]. The first solution implies the use of flexible and uncharged synthetic polymers (*e.g.* PEO and PVA) that *via* the hydrogen bonds formed between alginate and these polymers, decrease the repulsive force among polyanionic molecules and facilitate the chain entanglement [19]. Beside, Saquing *et al.* found that PEO favourably reduce surface tension, which facilitates electrospinning [14]. PEO is a unique class of non-ionic water-soluble biodegradable biopolymer, that due to its excellent biocompatibility, biodegradability and very low toxicity is proposed for the use in many biomedical applications. What is also noteworthy, PEO is approved by Food and Drug Administration [7, 13].

Figure 1 shows scanning electron microscopy images of electrospun fibers from solution with addition of PEO with four different average molecular weights as a carrier polymer.

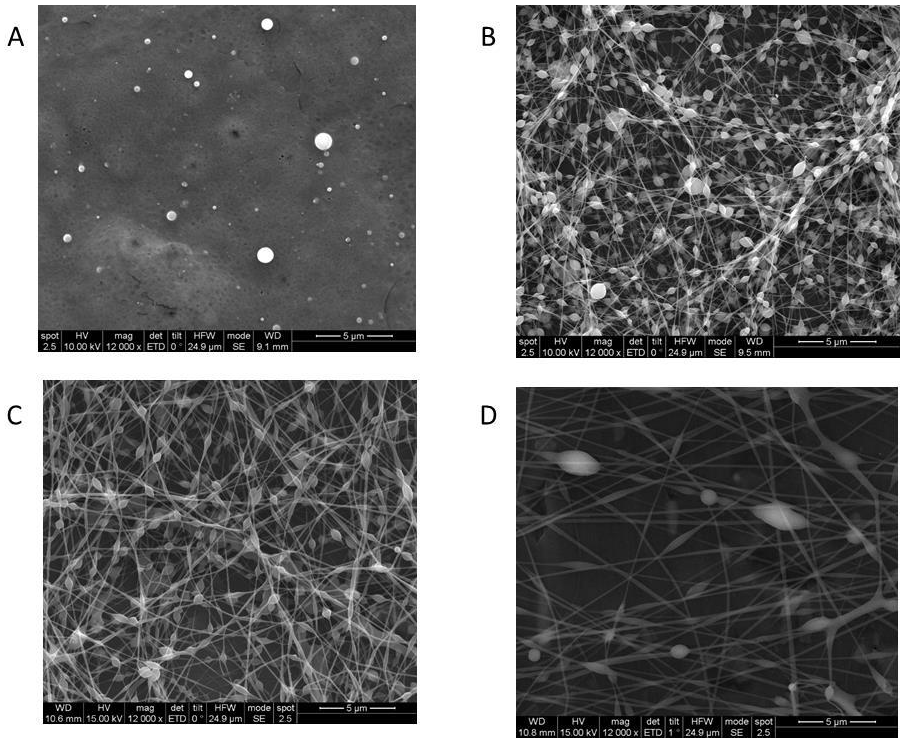

Figure 1. SEM images of electrospun alginate fibers: a) 2.0 wt.% AL, 2.0 wt.% PEO 100 kDa, b) 2.0 wt.% AL, 2.0 wt.% PEO 600 kDa, c) 2.0 wt.% AL, 2.0 wt.% PEO 1000 kDa and d) 2.0 wt.% AL, 2.0 wt.% PEO 2000 kDa.

The initial attempt at electrospinning of alginate-PEO fibers resulted in droplets or beaded fibers but no uniform nanofibers. Nevertheless, it was concluded that the higher molecular weight of the PEO better fibers with fewer beads are obtained. This is in agreement with work on the role of PEO as the "carrier polymer" in alginate-based nanofibers extensively studied by Saquing *et al.*. It was demonstrated that electrospining of alginate is only possible by blending with an appropriate polymer with a high molecular weight. It was speculated that PEO-PEO interactions of the high molecular weight producing sufficient chain entanglements in the resulting polymer blend solution play a key role in "carrying" the alginate from solution during electrospining. What is noteworthy, these interactions between carrier polymer and not those between the carrier polymer and alginate facilitate the electrospinnability of the blend solution [14].

Effect of surfactant on formation of electrospun alginate fibers

The second proposed solution of improvement of electrospinning from alginate solutions concerns addition of surfactant. In addition, the amount of alginate in the blend can be increased with the addition of small amounts of surfactant. A nonionic surfactant plays a significant role to the mat formation during electrospinning process, especially influences on morphology of fibers. Reducing the surface tension and the suppression of bead's defects are two of the most important tasks that surfactants meet [13]. Triton X-100 and Pluronic F-127 are the most commonly used surfactants in electrospinning of alginate-based nanofibers. Both have hydrophilic PEO blocks, however the hydrophobic polypropylene oxide (PPO) block from Pluronic F-127 is less toxic than the alkyl benzene block in Triton X-100. For this reason Pluronic F-127 is a more viable material for biomedical applications and it is a FDA-approved surfactant [13, 20].

The addition of small amounts of Triton X-100 or Pluronic F-127 to the sample generates bead-free fibers, as shown in Figure 2 (A-C). Only in cases of PEO with MW 2000 kDa with addition of Pluronic F-127 unfortunately it was impossible to obtain uniform nanofibers (Figure 2 F). Thus, in further study we decided to use PEO 1000 kDa.

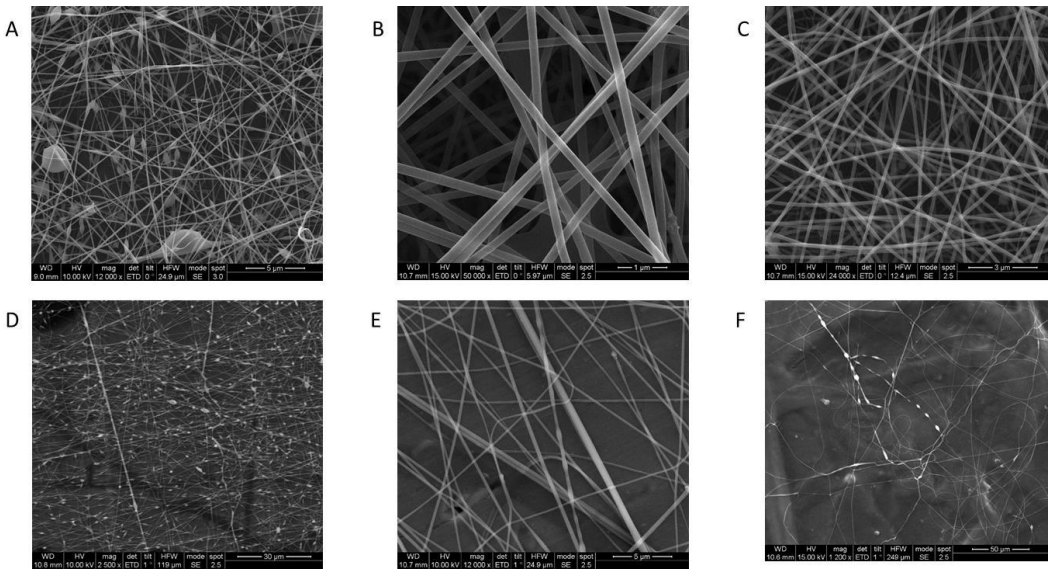

Figure 2. SEM images of electrospun a) alginate fibers without surfactant (3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa) and with addition of surfactant b) 0.5 wt.% Triton X-100, c) 0.5 wt.% Pluronic F-127 as well as d) alginate fibers without surfactant (2.0 wt.% AL, 2.0 wt.% PEO 2000 kDa) and with addition of surfactant e) 0.1 wt.% Triton X-100, f) 0.1 wt.% Pluronic F-127.

Since the potential use of alginate-based nanofibers is biomedical applications, there is a strong need to use only non-toxic reagents. Therefore in further study, we focused on application of Pluronic F-127 as a main surfactant, that effectively suppresses formation of bead defects in the investigated systems (Figure 3).

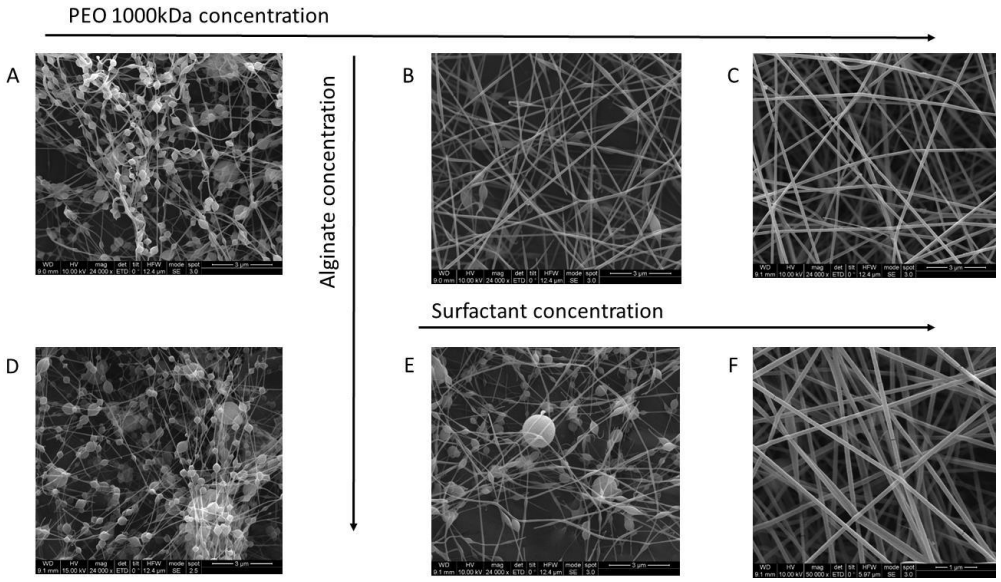

Figure 3. SEM images of electrospun alginate fibers: a) 3.0 wt.% AL, 1.5 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, b) 3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 0.5 wt.% Pluronic F-127, c) 3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, d) 4.0 wt.% AL, 1.5 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, e) 4.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 0.5 wt.% Pluronic F-127, f) 4.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127.

Free from beads and defects nanofibers with higher content of sodium alginate than additional polymer and surfactant have been successfully obtained only in case of 3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127 (Figure 3C) and 4.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127 (Figure 3F). They exhibited cylindrical shape and regular morphology with average diameter of 136±33 nm and 148±30 nm in case if lower and higher content of AL, respectively. Only simultaneous incorporation of the additional polymer and the surfactant resulted in nanofibers with good uniformity and structural integrity. Increase of only one of these components in blend alginate solution did not assure formation of bead-free fibers (Figure 3). Thus, both well-electrospinable polymer

(PEO) and a surfactant (in general, Triton X-100 or Pluronic F-127) have a significant effect on electrospinning processability of alginate nanofibers.

Our findings concerning the important role of surfactant in formation of uniform nanofibers with high content of alginate are in agreement with other authors [13, 14, 20]. For instance, Bonino *et al.* as well concluded that the addition of small amount of a surfactant (1.0 wt.%) effectively lowers the surface tension of polymer solution, supports formation of uniform nanofibers and enhances concentration of alginate and morphology of electrospun fibers from alginate-PEO blend solutions [20].

Influence of alginate concentration on alginate electrospun fibers formation

Furthermore, influence of increasing alginate concentration and constant concentration of Pluronic F-127 (1.0 wt.%) on formation of nanofibers with good uniformity and structural integrity has been investigated. SEM images of the electrospun fibers with increasing content of alginate are shown in Figure 4.

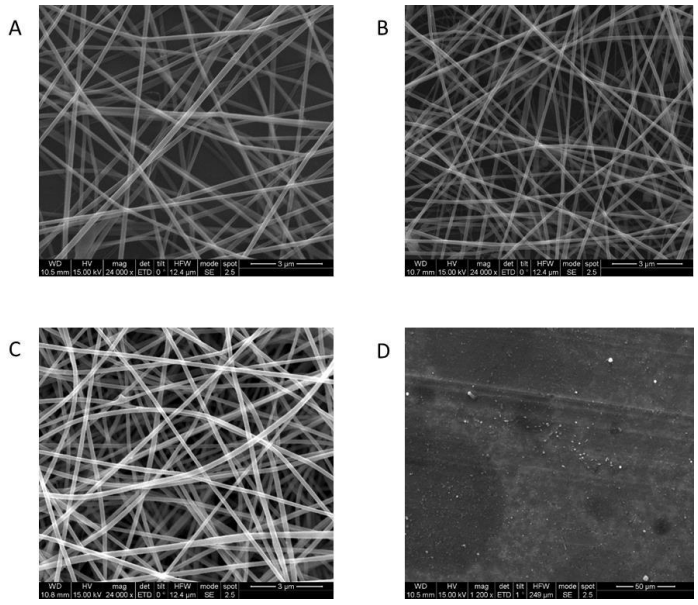

Figure 4: SEM images of different electrospun alginate fibers: a) 2.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, b) 3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, c) 4.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, d) 5.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127. (SIZES a) 125±29 nm b) 109±24 c) 177±50nm)

Smooth and uniform nanofibers were obtained in range of 2-4 wt.% of sodium alginate with constant concentration of PEO 1000 kDa (2.0 wt.%) and Pluronic F-127 (1.0 wt.%) (Figure 4A-C). Increased alginate concentration resulted in no significant trend in nanofiber sizes changes. Average size calculated for approximately 200 fibers was estimated to be 125±29 nm, 109±24 and 177±50nm for 2, 3 and 4.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, respectively. Increase of alginate concentration to 5.0 wt.% without changing of PEO 1000 kDa and surfactant content resulted in droplets formation (Figure 4D). It can be supposed that electrospinning was impossible because of high conductivity of alginate. This also confirms that addition of both PEO and surfactant is necessary to generate bead-free fibers.

Formation of alginate electrospun fibers loaded with ciprofloxacin hydrochloride

Mixing antibiotics in the polymer solution prior to electrospinning is an easy method to load large quantities of a drug into practically any polymeric nanofibers. However, there are disadvantages of the resulting systems, mainly the loaded drug tends to leach out rapidly form the fibrous mats in an aqueous solution. This effect is termed “burst release” and widely described in scientific literature [2].

Using the same procedure as described in the previous sections, alginate-PEO blended solutions with the addition of ciprofloxacin hydrochloride were electrospun (Figure 5).

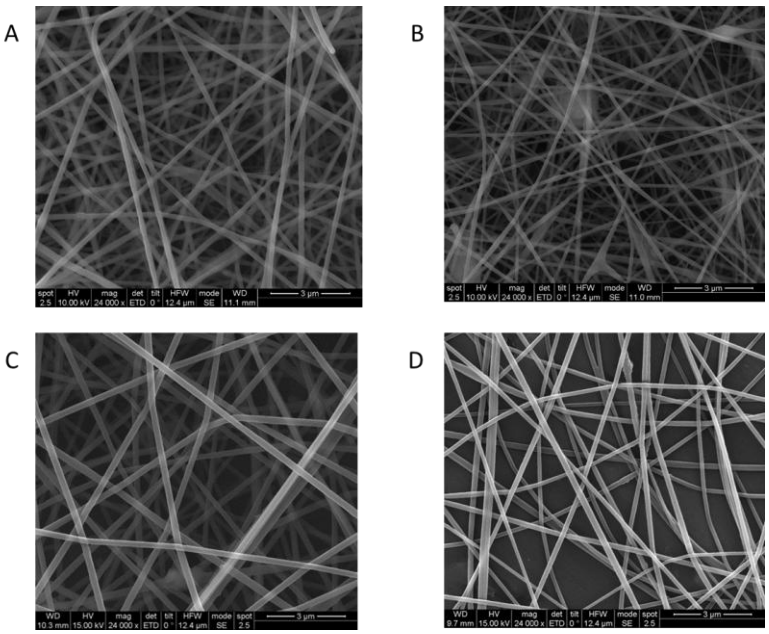

Figure 5. SEM images of different electrospun alginate fibers loaded with ciprofloxacin: a) 1.0 wt.% AL, 3.0 wt.% PEO 600 kDa, 1.0 wt.% Triton X-100, 0.2 wt.% CpHCl, (2.0 mg/ml) b) 2.4 wt.% AL, 1.6 wt.% PEO 600 kDa, 1.0 wt.% Triton X-100, 0.48 wt.% CpHCl,(4.8 mg/ml) c) 3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, 0.1 wt.% CpHCl (1.0 mg/ml) and d) 4.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, 0.1 wt.% CpHCl (1.0 mg/ml). (SIZES a) 103±22nm b) 86±20nm c) 119±36nm d) 161±32 nm)

The resulting nanofibers after addition of CpHCl were smooth and without any beads. It was concluded that the addition of the another component *i.e.* antibiotic to the electrospinning mixture did not changed significantly electrospinability of sodium alginate. It was possible to obtain uniform nanofibers with average size in range of 86-103 nm for 1 wt.% of alginate and 3 wt.% of PEO 600 kDa loaded with 2.0 mg/ml of CpHCl (103±22nm) as well as for 2.4 wt.% of alginate and 1.6 wt.% of PEO 600 kDa loaded with 4.8 mg/ml of CpHCl (86±20nm), both obtained with addition of Triton X-100 (1.0 wt.%) as a surfactant. While, in case of addition of PEO 1000 kDa (2.0 wt.% PEO) and Pluronic F-127 (1.0 wt.%) desirably smooth and flexible fibers were obtained with the average size of 119±36 nm and 161±32 nm for 3.0 and 4.0 wt.% AL, respectively.(why size of a and b differs significantly from c and d ????, discuss influence of the increase AL content)

In general, the morphology of electrospun nanofibers depends on solution parameters such as conductivity, viscosity, and surface tension [6]. In addition, when an aqueous drug solution is added into a polymer solution, the viscosity and surface tension of the mixed solution alter slightly, usually overall effect is negligible. Meanwhile, the conductivity of the solution can be increased by the presence of ionized drug molecules (although the exact value of increase in conductivity is not known), which increases the charge density of the jet resulting in the fibrous morphology. Indeed in our case, the addition of CipHCl could decrease the conductivity and its change decreased the average diameter of the resulting fibers without the influence on their morphological structure. As well; it did not affect solution conductivity, since electrospinning process was possible and resulted in formation of regular fibers. A possible explanation for the decreasing viscosity of the polymeric solution by the addition of CipHCl is that the drug could be trapped between polymeric chains, and thus acts as a plasticizer [9].

Post-treatment – stabilization and cross-linking

As expected, the as-prepared nanofibers were highly water-soluble and dissolved in water-based media. Therefore, to improve the stability of the electrospun mats they were immersed in ethanol for 1 min and then in ethanol solution of $CaCl_2$ for 10 min. Consequently, stabilization and cross-linking processes occurred in these conditions. The electrospun alginate fibers before and after post-treatment are presented in Figure 6.

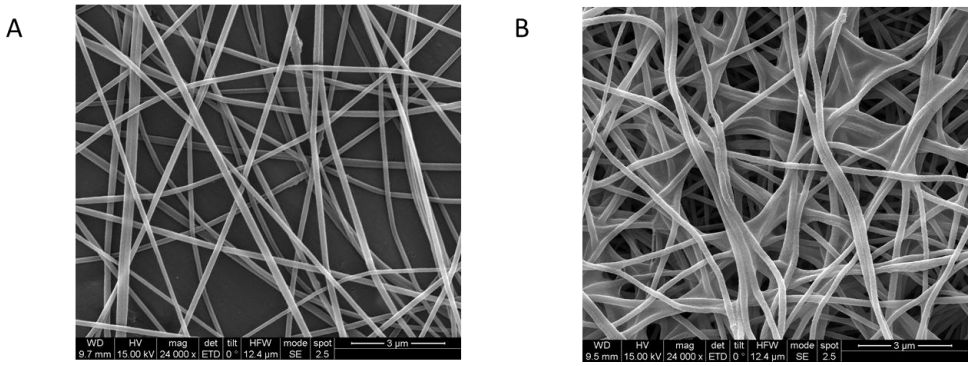

Figure 6. SEM images of electrospun alginate fibers loaded with ciprofloxacin 4.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, 0.1 wt.% CpHCl (1mg/ml) a) before and b) after stabilization and cross-linking processes. (SIZE: a) 161±32 nm b) 181±45 nm)

The applied post-treatment of alginate nanofibers had a negligible effect on their morphology. Furthermore, the fibers' diameter did not increased significantly after this procedure from 161±32 nm to 181±45 nm. It was concluded that the alginate-based nanofibers were successfully ionically cross-linked in a calcium solution without the need of the cytotoxic chemical cross-linkers application.

Loading efficiency

For nanofibers with 3.0 wt.% of alginate mixed with 2.0 wt.% of PEO 1000 kDa and 0.5 wt.% of surfactant (Triton X-100 or Pluronic F-127) it was possible to load even 1.0 mg/ml of CpHCl without any adverse effect on the structure, morphology and size of the resulting nanofibers (Figure 7).

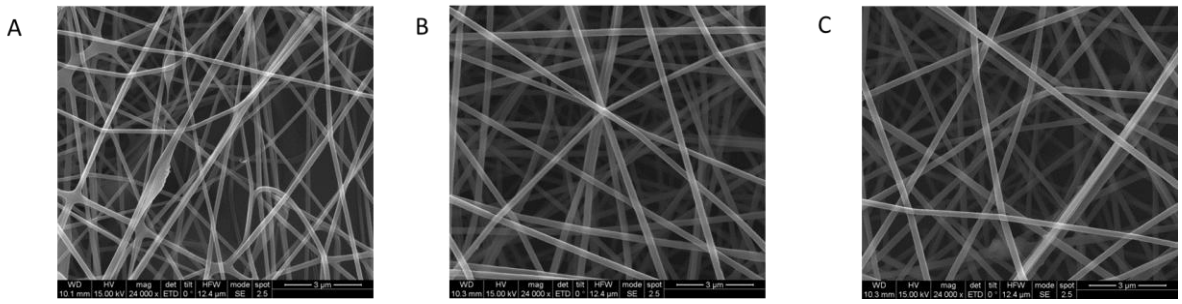

Figure 7. SEM images of electrospun alginate fibers loaded with different concentration of ciprofloxacin hydrochloride: a) 3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, 0.02 wt.% CpHCl (0.2 mg/ml), b) 3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, 0.08 wt.% CpHCl (0.8 mg/ml) and c) 3.0 wt.% AL, 2.0 wt.% PEO 1000 kDa, 1.0 wt.% Pluronic F-127, 0.1 wt.% CpHCl (1.0 mg/ml). (SIZES: a) 139±35nm b) 118±37 nm, c) 119±36nm)

The usual way of measuring it is to dissolve the polymers to release completely the drug and then measure it (by UV in this case).

Release of ciprofloxacin hydrochloride

In general, in case of fibers manufactured in such a way that the drug is mixed with polymer prior to electrospinning, the release of the agent in an aqueous environment is found to have a biphasic profile: an initial burst release followed by a much slower process thereafter. The high burst release can be ascribed mainly to the fact that the very small diameter and the high surface area in the nanofibers provide short diffusion pathway and are conducive to mass transfer of the drug [21].

For both *in vitro* and *in vivo* applications, fibrous biomaterials are expected to maintain the structural integrity in aqueous solution. Up to 15 days alg:peo 80:20 [7].

Conclusions

The resulting nanofibers are formed of high content of alginate, a natural biocompatible polysaccharide, as well as PEO and Pluronic F-127 (an ethylene oxide(EO)/propylene oxide (PO) block copolymer), both Food and Drug Administration approved polymers. This makes the resultant scaffolds promising for various biomedical

applications as it can be supposed that since the produced alginate scaffolds are free from cytotoxic chemicals and possess appropriate structural properties they can promote the attachment and proliferation of cells.

Acknowledgements

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Abbreviations

AL – sodium alginate

CpHCl – ciprofloxacin hydrochloride

FDA – Food and Drug Administration

PCL – polycaprolactone,

PLA – polylactic acid,

PGLA – poly(lactic-*co*-glycolic acid),

PEO – polyethylene glycol, poly(ethylene oxide),

PVA – poly(vinyl alcohol),

PVP – poli(vinylpyrrolidone),

ECMs – extracellular matrix,

References

[1] Stachewicz U, Qiao T, Rawlinson FCS, Veiga Almeida F, Li WQ, Cattell M, et al. 3D imaging of cell interactions with electrospun PLGA nanofiber membranes for bone regeneration. Acta Biomaterialia 2015.

[2] Fattahi P, Borhan A, Abidian MR. Microencapsulation of Chemotherapeutics into Monodisperse and Tunable Biodegradable Polymers via Electrified Liquid Jets: Control of Size, Shape, and Drug Release. Advanced Materials 2013;25:4555-60.

[3] Qi H, Hu P, Xu J, Wang A. Encapsulation of Drug Reservoirs in Fibers by Emulsion Electrospinning: Morphology Characterization and Preliminary Release Assessment. Biomacromolecules 2006;7:2327-30.
[4] Hajiali H, Heredia-Guerrero JA, Liakos I, Athanassiou A, Mele E. Alginate Nanofibrous Mats with Adjustable Degradation Rate for Regenerative Medicine. Biomacromolecules 2015;16:936−43.
[5] Ma G, Fang D, Liu Y, Zhu X, Nie J. Electrospun sodium alginate/poly(ethylene oxide) core–shell nanofibers scaffolds potential for tissue engineering applications. Carbohydrate Polymers 2012;87:737- 43.
[6] Lu JL, Zhu YL, Guo ZX, Hu P, Yu J. Electrospinning of sodium alginate with poly(ethylene oxide). Polymer 2006;47:8026-31.
[7] Bhattarai N, Li Z, Edmondson D, Zhang M. Alginate-Based Nanofibrous Scaffolds: Structural, Mechanical, and Biological Properties. Adv Mater 2006;18:1463-7.
[8] Lee KY, Jeong L, Kang YO, Lee SJ, Park WH. Electrospinning of polysaccharides for regenerative medicine. Advanced Drug Delivery Reviews 2009 61:1020-32.
[9] Unnithan AR, Barakat NAM, Pichiah PBT, Gnanasekaran G, Nirmala R, Cha YS, et al. Wound-dressing materials with antibacterial activity from electrospun polyurethane–dextran nanofiber mats containing ciprofloxacin HCl. Carbohydrate Polymers 2012;90:1786- 93.
[10] Watthanaphanit A, Supaphol P, Furuike T, Tokura S, Tamura H, Rujiravanit R. Novel Chitosan-Spotted Alginate Fibers from Wet-Spinning of Alginate Solutions Containing Emulsified Chitosan-Citrate Complex and their Characterization. Biomacromolecules 2009;10:320-7.
[11] Paneva D, Ignatova M, Manolova N, Rashkov I. Novel chitosan-containing micro- and nanofibrous maerials by electrospinning: preparation and biomedical application: Nova Science Publishers, Inc.; 2009.
[12] Zamani M, Prabhakaran MP, Ramakrishna S. Advances in drug delivery via electrospun and electrosprayed nanomaterials. International Journal of Nanomedicine 2013;3:2997-3017.
[13] Bonino CA, Efimenko K, Jeong SI, Krebs MD, Alsberg E, Khan SA. Three-Dimensional Electrospun Alginate Nanofiber Mats via Tailored Charge Repulsions. Small 2012:1-9.
[14] Saquing CD, Tang C, Monian B, Bonino CA, Manasco JL, Alsberg E, et al. Alginate−Polyethylene Oxide Blend Nanofibers and the Role of the Carrier Polymer in Electrospinning. Ind Eng Chem Res 2013;52:8692−704.
[15] Kataria K, Gupta A, Rath G, Mathur RB, Dhakate SR. In vivo wound healing performance of drug loaded electrospun composite nanofibers transdermal patch. International Journal of Pharmaceutics 2014;469:102-10.
[16] Sun J, Tan H. Alginate-Based Biomaterials for Regenerative Medicine Applications. Materials 2013;6:1285-309.
[17] Lee KY, Mooney D, J. Alginate: Properties and biomedical applications. Progress in Polymer Science 2012;37:106- 26.
[18] Gonen SO, Taygun ME, Kucukbayrak S. Evaluation of the factors influencing the resultant diameter of the electrospun gelatin/sodium alginate nanofibers via Box–Behnken design. Materials Science and Engineering: C 2016;58:709-23.
[19] Hu X, Liu S, Zhou G, Huang Y, Xie Z, Jing X. Electrospinning of polymeric nanofibers for drug delivery applications. Journal of Controlled Release 2014;185:12-21.
[20] Bonino CA, Krebs MD, Saquing CD, Jeong SI, Shearer KL, Alsberg E, et al. Electrospinning alginate-based nanofibers: From blends to crosslinked low molecular weight alginate-only systems. Carbohydrate Polymers 2011;85:111-9.
[21] Gao Y, Truong YB, Zhu Y, Kyratzis IL. Electrospun Antibacterial Nanofibers: Production, Activity, and In Vivo Applications. J APPL POLYM SCI 2014:40797-810.