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Electrospinning Alginate/Polyethylene Oxide Nanofiber Composites for Curcumin Delivery

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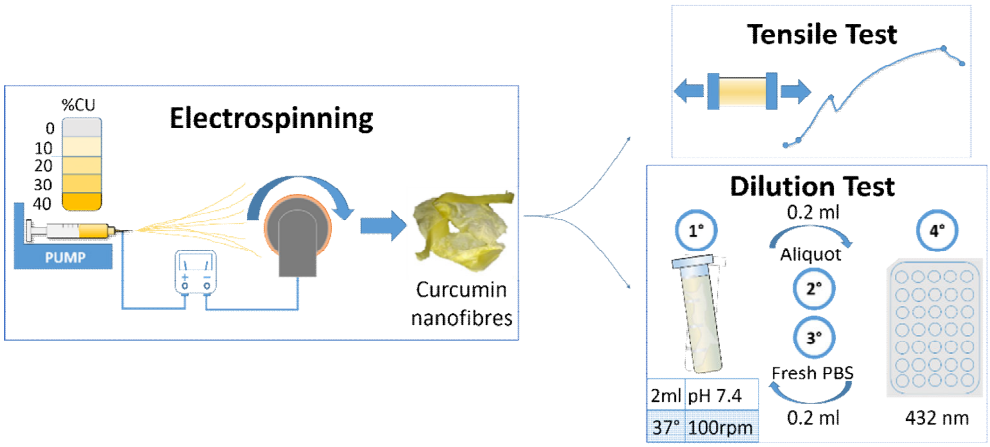
ABSTRACT: Manufacturing a sodium alginate (SA) and polyethylene oxide (PEO) composite loaded with antimicrobial curcumin (CU) was accomplished in this study. Crosslinked with trifluoroacetic acid (TFA), this nanofiber mat was mechanically characterized for the first time along with the morphological and release properties to deliver CU over a large range of CU concentrations from 10 to 40 wt%. Mechanical properties were affected by both CU addition and the cross-linking process, resulting in a higher ultimate tensile stress (from 4.3±1.9 to 15.1±1.7 at 10% CU) and Young modulus (7.6±2.8, 44±2.94 before and after TFA). CU was successfully encapsulated in the SA nanofibers and its delivery was effective over a 24 h period, demonstrating appropriate drug eluting mechanism. Excellent mechanical properties obtained with a biocompatible TFA crosslinking together with natural properties of alginate could turn this nanofiber composite into a great candidate for water filtering and for tissue engineering. Additionally, loaded with CU, it could also become an ideal drug delivery vehicle.

KEYWORDS electrospinning, sodium alginate, curcumin, nanofibers, biomedical



1. INTRODUCTION

Electrospinning (ES) of sodium alginate (SA) allows the production of nanofiber meshes and scaffolds with immense advantages such as a high surface area ideal for drug release and heavy metals adsorption [1]. Hence, ES of SA can be an excellent option for drug delivery and water filtering applications. Although challenging [2][3], electrospinning of SA has been extensively studied in the past. Due to the SA low gelling point, a high molecular weight copolymer like polyethylene oxide (PEO) [3], is added to the solution to form suitable chain entanglements, normally along with surfactants [3].

Another challenge when electrospinning SA is the spontaneous formation of 3D structures due to higher availability of anions from SA at high humidity (>Although it can be an opportunity to manufacture scaffolds with high porosity and cell infiltration properties, this has not been achieved completely up to date, and can be an issue for other applications20RH%) [4]. Therefore, a controlled

atmosphere using air conditioning is necessary, increasing costs, especially for high scale production.

In order to increase resistance of SA to water or aqueous body fluids, Hajiali et al. [5] suggested a biocompatible method that consisted in soaking the electrospun SA mats on trifluoroacetic acid (TFA), enabling better control of the degradability rate of the mats and overcoming drawbacks of previous methods [6] [7].

Curcumin (CU), an extract of the Curcuma Longa, is claimed to have efficacy in relation to a range of diseases including from anti-cancer to anti-oxidant and anti-arthritic behavior as well as having anti-microbial efficacy [8] [15].

In this study a biocompatible nanofiber system loaded with CU is manufactured and its main properties are explored. Tensile properties of TFA crosslinking are analyzed for the first time while an economical method is reported for electrospinning a homogeneous SA nanofiber under high humidity conditions. In addition, CU drug release was studied. This nanofiber composite could be of great interest for applications such as tissue engineering or filtering media.

2. EXPERIMENTAL SECTION

2.1 Materials. SA (<40 kDa), PEO (1.000 kDa), Pluronic® F-127 (PL), TFA, Phosphate buffer saline solution (PBS) were purchased from Sigma Aldrich. CU with a formulation of 200 mg of nanocurcumin and 2 mg of Bioperin® was acquired from Triso®.

2.2 Preparation of solution for electrospinning. Two separate solutions of 8 wt% of SA and 4 wt% of PEO were prepared solubilized in deionized water, during 4h and further mixed together in the proportions 25:9.5 (g SA: g PEO). Subsequently, Pluronic® F-127 was added at 2 wt% of the whole solution. Final dry mat resulted in an 84 wt% of SA. To prepare the CU solutions, a saturated solution of CU and water was added to a SA and PEO solution of higher concentration, ending with the same SA/PEO/solvent specified before. Final loadings were 10, 20, 30 and 40 wt% CU (wt% CU/wt% polymer).

2.3 Electrospinning of curcumin loaded SA. A non-sterile 5 ml Luer lock PE syringe was loaded with the solution (4 ml), using a blunt needle with 3 cm length and 23 gage needle, and introduced in the ES machine (LE-50 FLUIDNATEK™ eSpinning tool, Bioinicia). The needle to collector distance was kept at 15 cm, using the rotator drum at 700 rpm. The applied voltage was varied within 15-23kV while the feed rate stayed at 0.3-1 mL/h. All fibers were obtained at room temperature (21-25°C) and 40-50% of relative humidity. The ES time for all the mats was 3 h, and they were peeled off directly from the drum.

2.4 Cross-linking of the mats. The cross-linking method used in this project is described by Hajiali et al. [5]. Squares of 9 cm^2 were cut and immersed into the TFA for 24h and left for drying during further 24h inside the fumehood.

2.5 Characterization of fibers. A XL30 (FEI) Environmental Scanning Electron Microscope (ESEM) was used to study fiber morphology, diameter size and directionality. Images were processed with ImageJ and DiameterJ. Data

was treated with the fitting software Fityk® when the diameter distributions were not normal.

2.6 Mechanical testing. Strips of 26x12 mm^2 were cut from the electrospun mats with a roller-cutter and glued on paper on its ends using a Deben MT200 microtensile machine with a 5N load cell, 1 mm/min speed and a separation grip of 10mm. Tensile strength values were calculated dividing the force values by the cross-section and the thickness of the specimens were obtained with a Dektak stylus profilometer. The tests were done at room temperature and at least five specimens were prepared per type of sample.

2.7 Drug release. The experiments were conducted using PBS with pH 7.4, 37°C and stirring at 100 rpm. At least 3 fiber mat replicates of every CU concentration were tested on disposable 2 ml containers for 4 days. After, 200 µL were extracted each time from the stock analyzed on the spectrophotometer at 432 nm and replaced with fresh PBS to maintain sink conditions. Cumulative percentages were calculated based on the total amount of curcumin released.

2.8 Statistical analysis. Data was analyzed with an ANOVA test and a TukeyHSD post-hoc treatment, considering $p<0.05$ as statistical different and results were reported as average (M) ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Fibers morphology

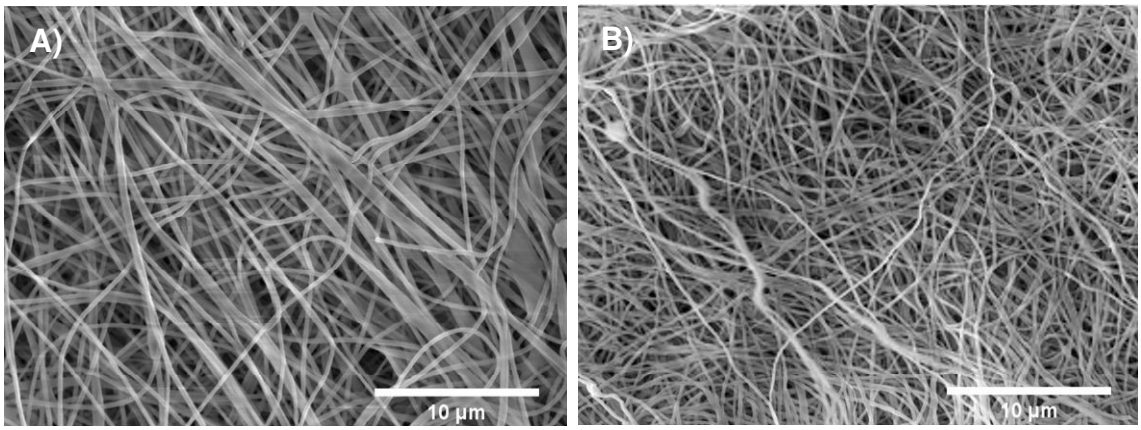


Figure 1. SEM images. A) SA/ PEO nanofibers. B) SA crosslinked with TFA nanofibers

The final electrospun mat containing 84 wt% of SA is shown on Figure 1A together with the crosslinked version (100% alginic acid). Attending at Figure 1B, the nanostructure is seamlessly maintained showing wavy fibers in contrast. Furthermore, the diameter decreases 15% (from 250 to 212 nm), almost matching the original PEO content (16%). It is worth noting that only CU encapsulated by PEO is lost during cross-linking, as CU is not soluble in acid environments [10].

Because of the high humidity during the experiments, 3D structures were built-up on the static collector in accordance to previous works [4]. Hence the spinning collector was used at minimum speed, so an acceptable uniformity of the mat was achieved. Due to the rotating movement, fibers are dragged on the tangential speed direction, helping to coat the cylinder, instead of contributing to agglomeration.

On Figure 2 SEM images of the different CU loaded mats are displayed together. The final mats are shown as well on the insets. On Figure 2A, fibers can integrate 10% of CU with no apparent defects. In the case of 20% CU (Fig. 2B), some defects are present such as few CU particles and a prominent joining of several fibers. With 30% CU (Fig. 2C) the formation of fibers is affected leading to the formation of beads. At this point, the SA mat is totally saturated being unable to integrate all the CU as visible from the micron sized particles on Figure 2C. Finally, nanofiber formation is totally disrupted at 40% CU (Figure 2D). Indeed, a uniform layer spreads all over with some micron-sized fibers.

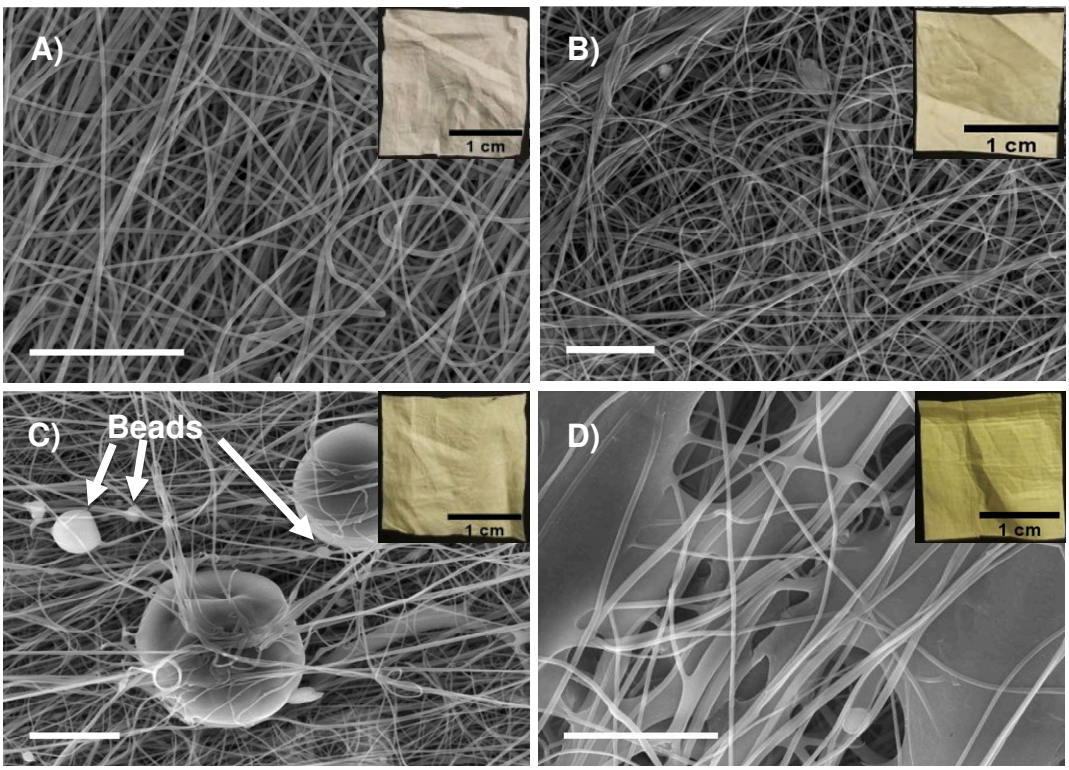


Figure 2. SEM images of the SA/PEO mats. A) 10% CU. B) 20% CU. C) 30% CU. D) 40% CU. (All scale bars 10 µm).

3.2 Mechanical testing.

The main mechanical properties of the mats, such as ultimate tensile stress (UTS), elongation at break and Young modulus (E) are summarized on Table 1. Stress/strain curves can be consulted on the SI. Comparing samples before and after TFA and no CU loading, its E (MPa) shows a great increase from 7.56 to 43.55 MPa, $F(1, 9) = 11.9$, $p<0.01$, that is, almost six times larger than after the treatment, adducing a great increase of strength in the elastic region. This is probably caused by the suppression of the PEO after the cross-linking process which can act as a plasticizer [11]. This strong improvement exceeds other attempts in the literature [12] [13].

Analyzing the CU loaded samples (before and after TFA), similar effects are encountered, obtaining increments of UTS and E in accordance with other electrospun mats loaded with CU [14]. In the case of the E, strong increment (~100%) at 10% CU is lost for subsequent loadings until it recovers at 40% CU, suggesting that CU addition can be detrimental at high doses. Results at 40% could be explained due to the loss of the nanostructure of the mat (Figure 2D), having more effective area to withstand the tensile force. Elongations at break increase at 10% (+4.71%) and 20% (+2.97%) but further increments of CU are negative, having results non-statistically different from non-loaded samples, that is a decrease of its plasticity. Finally, comparing the UTS values, small amounts of CU (10% CU) can be beneficial for the tensile strength increasing from 4.34 to 7.05 MPa, but further increments of CU turn out in a detrimental effect, probably due to more concentration of defects, obtaining even smaller UTS at 30% CU (4.30 MPa), while for 40% CU samples there is not a statistical

difference regarding non-loaded samples. The magnitude order of the experimented electrospun mats are in the range of a few megapascals in consonance to other studies [12][15]. Nevertheless, after TFA crosslinking very significant results are achieved exceeding other studies.

3.3 CU release

On Figure 3, it is gathered all the data regarding the relative CU release profile. According to the results, similar eluting mechanisms were experienced regardless of the concentration, as deducted from similar release profiles. Thereby, initial strong release of 20-33% of the CU on the first hour is followed by a slow release mainly during the next 23h. Because CU is embedded in the nanofibers or placed on the surface as seen on the SEM results, there are at

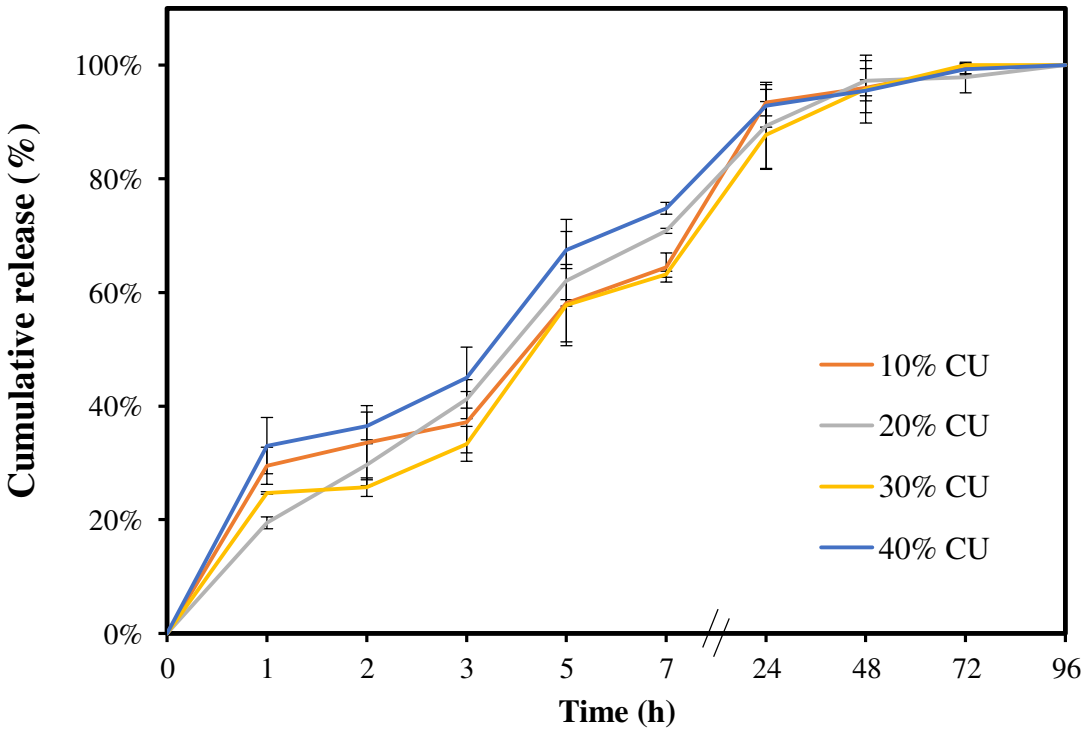


Figure 3. CU release profile of cross-linked electrospun mats loaded with different %CU loadings.

least three possible mechanisms by which CU can be released. By diffusion, polymer degradation or dissolution of the CU covering the surface. CU on the surface is normally related to a burst release. However, this possibility is not a dominant fact as slow initial release and the similarity between 10 and 40% CU samples reveals. This situation is likely to be due to limited solubility of the CU in the PBS media [16]. In the case of freely water-soluble drugs, drug on the surface would automatically dissolve, once in contact with the media. Alternatively, poorly water-soluble drugs cannot be dissolved and remain stable [16]. Likewise, the majority of the CU is released before 5h, this suggests that diffusion is the main mechanism of release, because nanofibers take longer to dissolve and there is no burst release [5]. In order to confirm the validity of this method 0% CU samples were analyzed in the same way registering 2 mg of CU relative to an initial weight of 23 mg of the mat, that is an 8% of the mat weight, suggesting that only a small amount of the degraded mat is participating in the absorbance readings. This is indicative of the accuracy of the method, probably overestimating the CU readings although in a small extent.

CONCLUSIONS

Nanofibers made of SA-PEO were successfully obtained incorporating CU with potential biomedical and water filtering applications. Mechanical properties were affected by CU addition and the TFA cross-linking, resulting in higher tensile stress and E at the expense of the elasticity and plasticity of the final mat. Electrospun nanofibers showed correct incorporation of CU, demonstrating

appropriate drug elution over a 24h period without initial burst release.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at XXXXXXX.

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Table 1. Mechanical properties of electrospun mats before (left) and after (right) TFA treatment at different CU loadings (M ± SD) (*).

	Before TFA					**After TFA**				
CU loading	**0%**	**10%**	**20%**	**30%**	**40%**	**0%**	**10%**	**20%**	**30%**	**40%**
E (MPa)	7.6±2.8	15.1±1.7	11.9±2.9	7±4	16.8±5	44±2.94	32±3.4	18.2±6.7	36±13	37.5±2
σ_{UTS} (MPa)	4.3±1.9	7.1±0.7	4.9±1	4.3±1.4	5.8±0.5	5.9±2	9.1±0.94	7.2±0.9	7.9±2.7	10±2
E_T (%)	10.1±1	10.6±0.9	10.4±1.5	9.9±1.7	8.7±2.4	7.9±3	7.4±1.2	9.2±3.7	6.5±5	4.5±2

E: Young modulus

σ_{UTS}: Ultimate tensile strength

E_T (%): Elongation at break percentage

(*) For more data information see SI

Electrospinning alginate/polyethylene oxide and curcumin composite nanofibers

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