DOUBLE-FUNCTIONALIZED CHITOSAN NANOFIBERS FOR WOUND HEALING

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1. INTRODUCTION

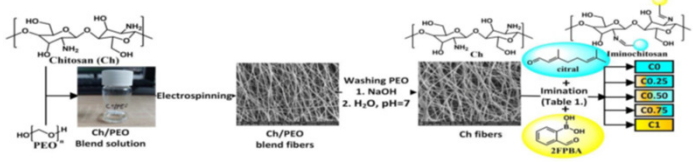
Many materials were proposed and investigated as dressings for wound management. Among them, chitosan based nanofibers demonstrated a high potential for wound healing [1]. Their composition and structure mimic the body's own extracellular matrix favoring the tissue regeneration, while their fibrous nature guarantees for an easy application on wounds, gas exchange and exudate drainage while maintaining a moisture environment [2]. Moreover, the intrinsic properties of chitosan, such as biocompatibility, biodegradability, hemostatic and antimicrobial activity are good premises for wound healing [3]. Besides, the biodegradation products of chitosan, which are natural metabolites, should favor the healing process [4].

In this context, a series of functionalized chitosan nanofibers was prepared, using two aldehydes with complementary bioactivities, one having strong antimicrobial activity (2-formylphenylboronic acid, coded 2-FPBA) and the other one (citral) having potential to enhance it by improving the cell permeability, presenting also antimicrobial activity by itself. The aldehydes were attached to chitosan nanofibers by imine linkages, which are reversible in water, favoring the aldehydes' gradual release under external stimuli, such as: moisture, pH, aldehyde consuming/leakage. In such a way, the fibers can gain self-defense properties against pathogens.

2. MATERIALS AND METHODS

2.1 The functionalized fibers obtaining

The functionalized fibers were prepared in three steps: (i) electrospinning of a CS/PEO blend; (ii) removing the PEO from fibers to yield neat CS fibers; (iii) functionalization of the neat CS fibers by condensation with the two aldehydes: 2-formylphenylboronic acid and citral (Scheme 1).



Scheme 1. Representation of the experimental pathway for nanofibers' obtaining

The condensation reaction was realized in heterogeneous system, by immersing the CS fibers into the solution of the two aldehydes in dry ethanol.

Table 1. Reaction parameters and samples' codes.

Code	C0	C0.25	C0.5	C0.75	C1
$NH_2/CHO_{2FPBA}/CHO_{citral}$ molar ratio	1/0/1	1/0.25/0.75	1/0.5/0.5	1/0.75/0.25	1/1/0

2.2 Functionalized chitosan nanofibers characterization

- The structural characterization: FTIR & NMR spectroscopy
- The morphology & supramolecular structure: SEM & POM
- Dynamic character: UV–vis
- *In vitro* biocompatibility on fibroblasts: MTS assay
- Antimicrobial activity: disk diffusion assay

3. RESULTS & DISCUSSIONS

3.1 Structural characterization by FTIR and NMR spectroscopy

The FTIR spectra confirmed that both aldehydes reacted with the amine groups of chitosan forming imine units, by the presence of their characteristic absorption bands, 1644 cm^{-1} for citryl-iminochitosan derivative and 1624 cm^{-1} for 2-FPBA-iminochitosan one (Fig.1 a). NMR spectroscopy confirmed the FTIR data, by the presence of the chemical shifts corresponding to the imine protons from the iminochitosan derivatives between 8.4 and 8.8 ppm (Fig 1b).

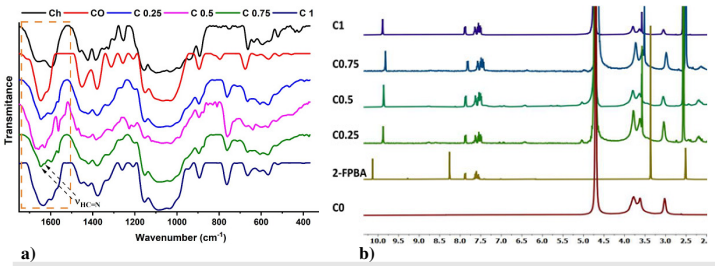
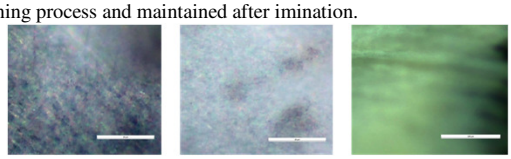


Fig.1 FTIR spectra (a) and NMR spectra (b) of the functionalized chitosan fibers and references

3.2 Polarized optical microscopy

POM images of the functionalized chitosan nanofibers displayed a birefringent texture, indicating a highly ordered supramolecular architecture gained during the electrospinning process and maintained after imination.

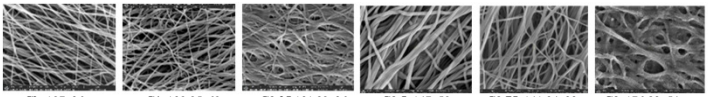


C0.25 C0.75 C0

Fig. 2 Representative POM images of the studied samples (scale: 20 µm)

3.3 Scanning electron microscopy

The functionalized fibers had lower diameter values than neat chitosan ones (Fig. 3), indicating the collapsing of the intra-fiber pores during the drying process.



Ch, 197±36 nm **C1**, 129.95±69 **C0.25**,131.29±26 **C0.5**, 147±59 **C0.75**, 141.24±33 **C0**, 176.82±54

Fig. 3 SEM images of the studied fibers and their average

3.4 Dynamic character of the imine units

A comparative analysis of the aldehydes release from **C1** and **C0** samples revealed a faster kinetics for 2FPBA (hydrophilic) compared with citral (hydrophobic), while the other samples (**C0.25**; **C0.5**; **C0.75**) presented intermediate values (Fig. 4).

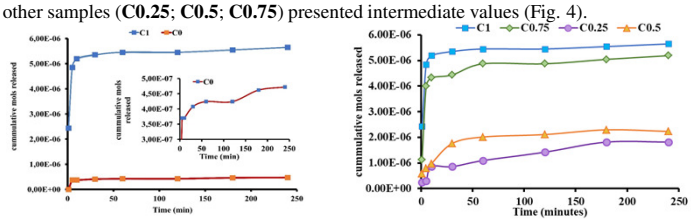


Fig.4 a) Cumulative aldehyde released in 24 h b) Cumulative 2FPBA released in 24 h

3.5 *In vitro* biocompatibility on fibroblasts

All the synthetized functionalized nanofibers preserved the cell viability at values higher than 80%, indicating their safe use for in vivo applications.

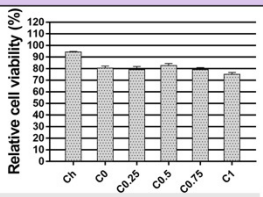


Fig.5 Results of biocompatibility tests of the nanofibers with MTS assay

3.6 Antimicrobial properties

All the samples presented strong antimicrobial activity against 5 microorganisms, the diameter of the inhibition zone, reaching the highest value of 25.9 mm against *A. brasiliensis* for the sample **C1** (Fig.6).

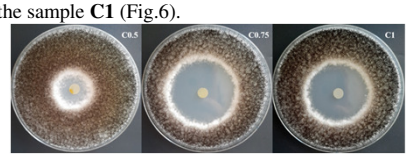


Fig.6 The inhibition zones of the samples against *A. brasiliensis*

4. CONCLUSIONS

Novel dynamic biomaterials were prepared by double imination reaction of chitosan nanofibers with two aldehydes: 2-FPBA and citral. The systems were biocompatible, as demonstrated by the *in vitro* tests on fibroblasts and presented high antimicrobial activity against 5 microorganisms, presenting high potential for being used in the development of wound dressing materials.

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ACKNOWLEDGEMENTS

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