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NANOFIBERS VERSUS HYDROGELS AS COLLAGENASE CARRIER

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ABSTRACT
Collagenase acts by promoting wound debridement, contributing to the tissue repair process. Several studies pointed collagenase as a substance involved in the elimination of devitalized tissue or any contaminated material found in the wound bed after the appearance of a lesion or skin burn. In the present work, hydroxypropyl methylcellulose/cyclodextrins hydrogels and polycaprolactone nanofibers with the ability to transport and release collagenase for the treatment of skin lesions, were synthesized and characterized. The collagenase polymeric carriers showed good physicochemical properties and presented the ability to retain the enzyme in its structure. Controlled release studies revealed a slow release of active collagenase confirming the ability to be used as carrier devices in the treatment of skin lesions.

MATERIALS AND METHODS
Polycaprolactone (PCL) nanofibers were produced by the electrospinning technique. Different conditions in terms of syringe diameter, magnetic field, distance from slider to fixed manifold, syringe outlet flow rate, syringe cleaning time, and fixed manifold deposition time were tested and the nanofibers production was optimized. The collagenase was incorporated in the polymer solution. Hydrogels based on hydroxypropyl methylcellulose (HPMC) and cyclodextrins (βCD or HPβCD) were synthesized according to the previous description by our research group [1]. The collagenase was incorporated by adsorption in the hydrogels. The concentration and activity of the collagenase was determined using a synthetic substrate, FALGPA. This substrate was hydrolysed by the collagenase retained in the polymeric structures [2]. The swelling sensibility of the polymeric carriers to different environmental pH conditions was studied. The developed materials were characterized by FTIR, DSC and TGA techniques. For the PCL nanofibers, a microscopic analysis was also performed using a scanning electron microscope (SEM).

RESULTS AND DISCUSSION
The properties of nanofibers and hydrogels were evaluated in terms of morphology, chemical structure, swelling behaviour and release profiles. Their morphological properties were observed by SEM. The nanofibers were smoothly round and presented a high surface area, with diameters between 195 – 200 nm and 110 – 137 nm for the fibres with and without collagenase, respectively (figure 1). Hydrogels possessed mechanical toughness with a hydrophilic surface covered with a highly porous matrices essential to acquire water in their interior.
Its swelling capacity was assessed in different pH conditions, with the HPMC-HPβCD hydrogel showing the highest water retention in all studied conditions.
The results showed that the incorporation of collagenase into the hydrogels by adsorption resulted in

a retention around 85.37% for the HPMC-HPβCD hydrogel and 84.15% for the HPMC-βCD hydrogel. The mechanism associated with the collagenase incorporation/retention in the hydrogel network was not exclusive of the hydrogels' swelling capacity.
The release of collagenase from the polymeric structures was evaluated. The cumulative amount of activity detected over time showed a slow release of the enzyme. After 24h, only 0.5% of the retained enzyme was released from the HPMC-HPβCD hydrogel, while for the HPMC-βCD hydrogel this value was around 0.18%. Given that the HPMC-HPβCD hydrogel swelling was higher than the HPMC-βCD swelling, and also due to the contribution of hydroxypropyl groups in the pore opening of the hydrogel, these results seem to be justified. In the case of PCL nanofibers, only a residual amount of the enzyme (0.05%) was released from the nanofibers at the end of 24h.

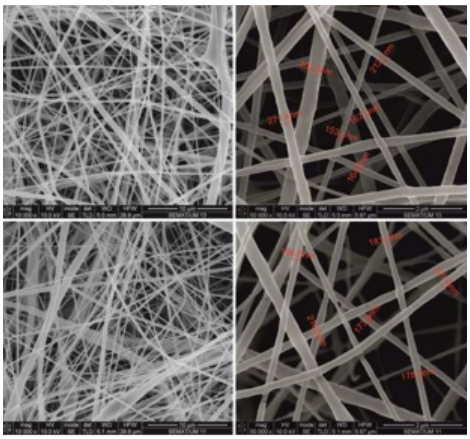

Figure 1. Nanofibers with collagenase

CONCLUSION

HPMC and CD hydrogels and PCL nanofibers with and without collagenase were developed and characterized by FTIR, DSC, TGA and SEM techniques.
The HPMC-HPβCD hydrogel showed the highest water retention in all the studied conditions. On the other hand, the HPMC-βCD hydrogel was the least sensitive to pH variations. However, both hydrogels presented similar enzymatic adsorption. The polymeric structures showed a high load of active collagenase. In the case of nanofibers, the slow-release profile of the enzyme in aqueous medium at room temperature confirms that collagenase is strongly retained in the matrix.

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