Development of a novel coaxial wet-spinning method to produce AAPV-loaded fibers for chronic wound care

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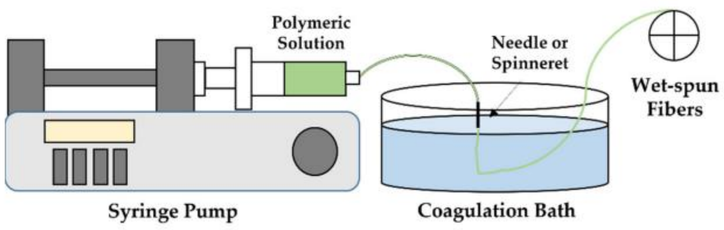
Introduction

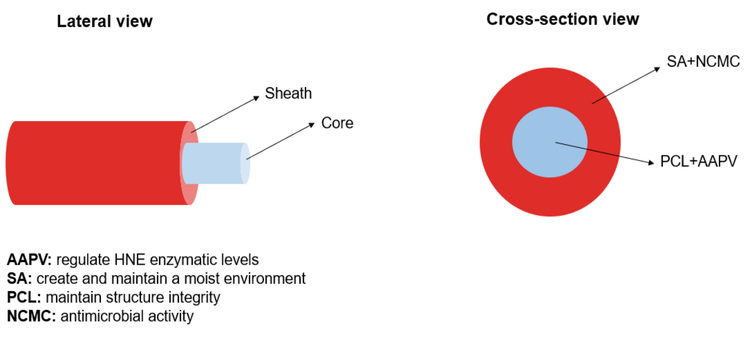
Chronic wounds (CW) are a worldwide concern, affecting a vast portion of the population, and compromising the health and quality of life of patients. The tetrapeptide Ala-Ala-Pro-Val (AAPV) has the ability to inhibit the activity of the enzyme human neutrophil elastase (HNE), which levels, in case of excessive inflammatory processes, remain abnormally high. Incorporation of peptides within polymeric structures (e.g. coaxial fibers) is very attractive to protect the payload from the surrounding environment and allow its controlled release for a sustained action. To this end, we proposed to engineer coaxial wet-spun fibrous structures loaded with the AAPV peptide. This system was designed to serve as a new delivery platform capable of a controlled and stepwise release of its content following pH-trigger, that not only fights infections but, most importantly, restores local enzymatic activity to normal levels. The outer layer (sheath) of the microfibers was made from blends of sodium alginate (SA) and N-carboxymethyl chitosan (NCMC), a chemically modified version of chitosan, responsive to basic pH (characteristic of CW) and endowed with antimicrobial action. Whereas the inner layer (core) was constituted by polycaprolactone (PCL) combined with AAPV. Polymers were selected based on their biocompatibility, biodegradability and spinnability. To the authors knowledge, this is the first report on coaxial wet-spun systems loaded with AAPV for CW care.

Wet-spinning

Technique based on a non-solvent-induced phase inversion process, including a polymeric solution extrusion into a coagulation bath composed by a poor solvent or a non-solvent/solvent mixture to form a coagulating filament that will solidify as a continuous polymeric fiber



Co-axial wet-spun fibers

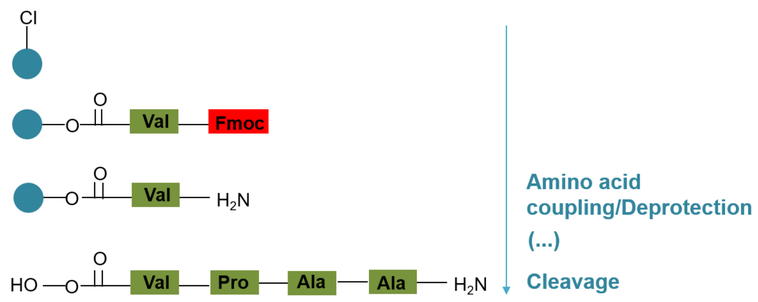


AAPV: regulate HNE enzymatic levels
SA: create and maintain a moist environment
PCL: maintain structure integrity
NCMC: antimicrobial activity

Produced fibers:
- **SA hollow** (core: coagulation bath; shell: SA);
- **SA-NCMC hollow** (core: coagulation bath; shell: SA combined with NCMC);
- **PCL** (core: PCL; shell: coagulation bath);
- **PCL-AAPV** (core: PCL combined with AAPV; shell: coagulation bath);
- **SA-PCL** (core: PCL; shell: SA);
- **SA-PCL-AAPV** (core: PCL combined with AAPV; shell: SA);
- **SA-NCMC-PCL** (core: PCL; shell: SA combined with NCMC);
- **SA-NCMC-PCL-AAPV** (core: PCL combined with AAPV; shell: SA combined with NCMC)

AAPV Synthesis and HNE Evaluation

Microwave-assisted solid-phase peptide synthesis technique

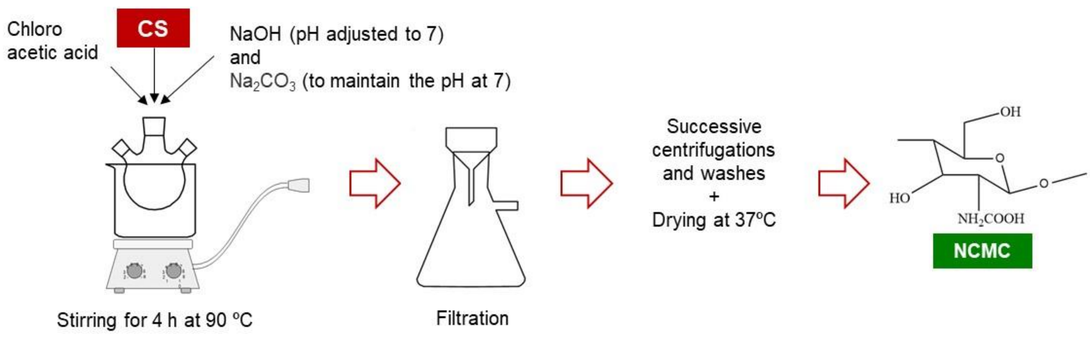


Evaluate AAPV's action towards HNE's enzymatic levels

Testing Substrate:
- Tris-HCl-buffer
- N-MeO-Suc-Ala-Ala-Pro-Val-p-NA

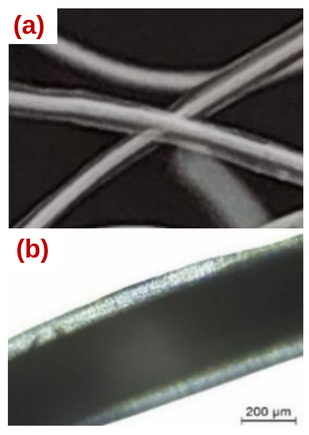
AAPV + HNE (Fibers incubated in Phosphate Bovine Solution (PBS)) → Soybean tripsin inhibitor solution (**to stop the reaction**) → Spectroscopy readings (**405 nm**)

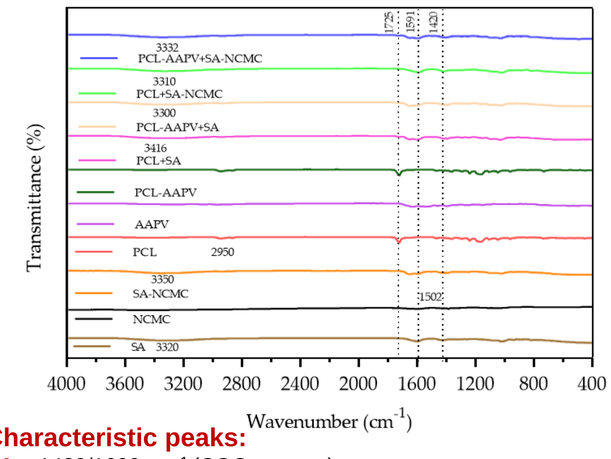
NCMC Synthesis



Fibers morphology and chemical characterization

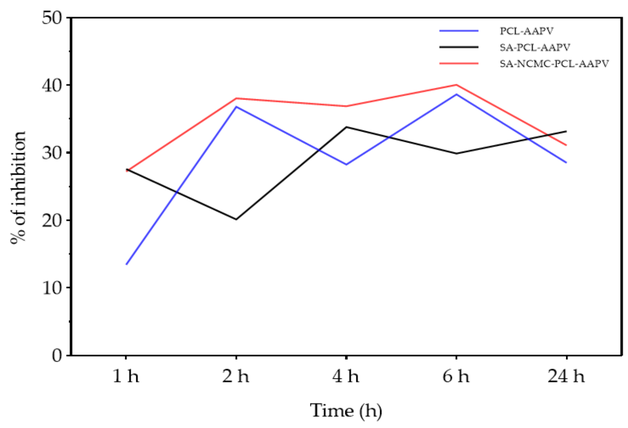
Figure 1. Macroscopic (a) and microscopic observations of co-axial wet-spun fibers





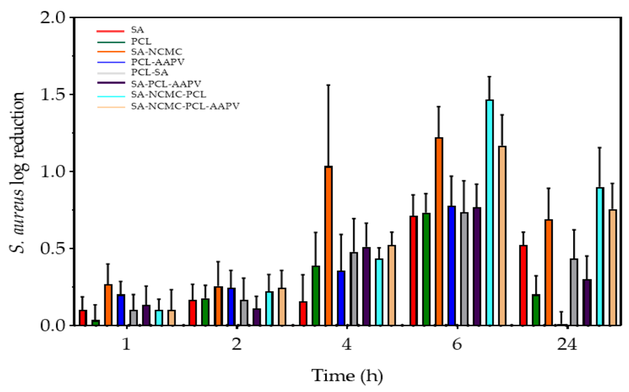
Characteristic peaks:
SA – 1420/1600 cm^{-1} (COO^- groups)
PCL – 1725 cm^{-1} (C=O groups)

Inhibition of HNE activity



t = 1 h: lower inhibition of HNE, due to a lower release rate of AAPV
t [2,24 h]: higher and constant release of AAPV and HNE inhibition
SA-NCMC-PCL-AAPV fibers presented a more constant HNE inhibition

Antimicrobial activity



NCMC-loaded fibers presented highest log reduction;
S. aureus activity was reduced at 90-99% at 6 h target

Conclusions

The potential of the engineered co-axial fibers to serve as controlled release platforms for NCMC was demonstrated, along with their inhibitory effect of HNE and antibacterial activity against *S. aureus*. Data confirmed the potential of this system to function as a stepwise, pH-triggered delivery platform, suitable for wound healing applications. With this investigation, a step further was taken in establishing wet-spun constructs for drug delivery in CW care.

Acknowledgments

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