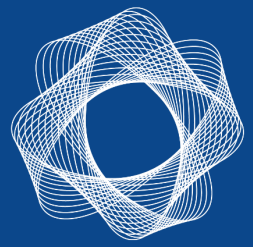

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Coaxial wet-spun fibers loaded with enzyme-inhibiting peptide for chronic wound care

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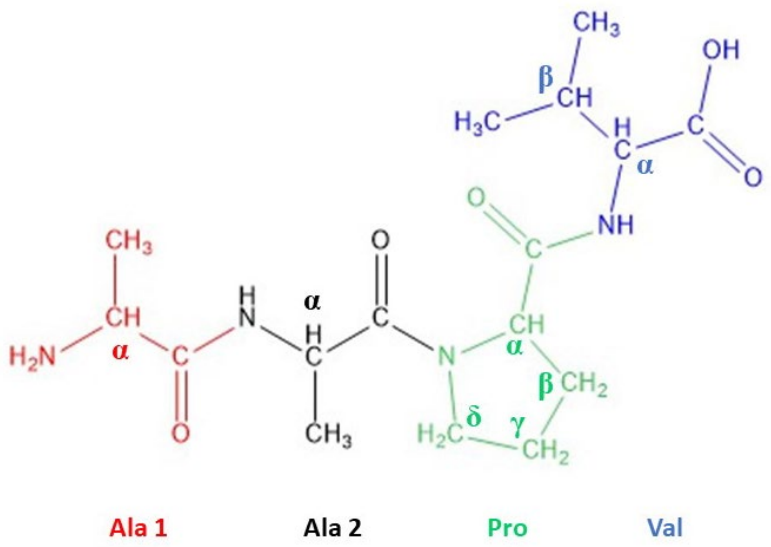
Antimicrobial Peptides

Antimicrobial Peptides

- Low MW molecules
- Composed of amino acid residues
- Control microbial proliferation
- Modulate immune response



Alanine-Alanine-Proline-Valine (Ala-Ala-Pro-Val or AAPV)

Inhibits human neutrophil elastase (HNE), which levels are abnormally high in the case of inflammatory processes related to CWs.

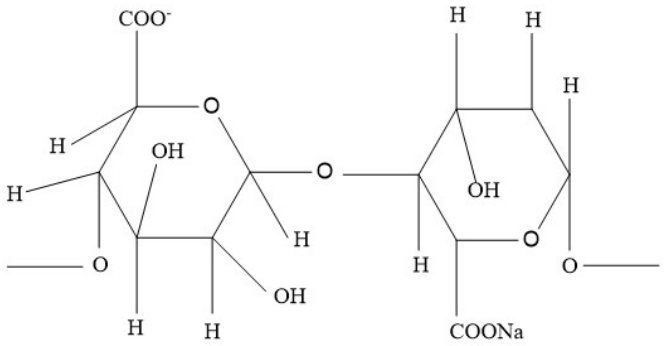
Low stability in physiological media

Incorporation within polymeric structures

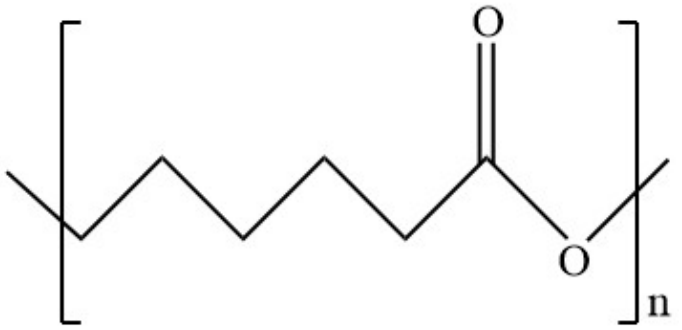
Santos, F.C. et al. Amino Acids, 49 (2017).

Polymers

Sodium alginate (SA)



Polycaprolactone (PCL)



Biocompatible; Biodegradable

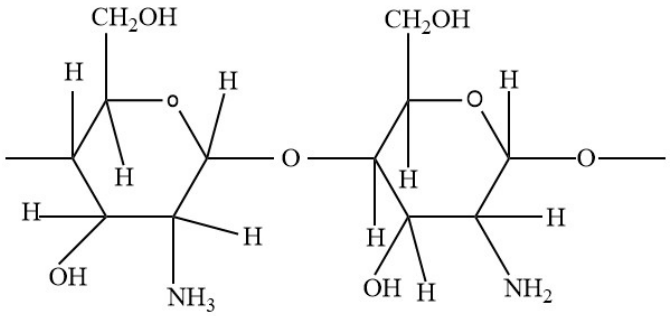
- Antiseptic properties;
- Water absorbent (elevated swelling capacity);
- Creates a moist environment.

- Synthetic polymer;
- Excellent mechanical properties;
- Slow degradation rate.

Plotczyk, M. et al. Elsevier. 1 (2019)
Eskandarinia, A. et al. Scientific Reports. (2020)

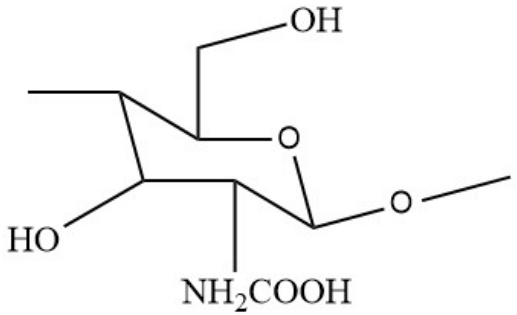
Polymers

Chitosan (CS)



Chemically modified

N-carboxymethyl chitosan (NCMC)



Biocompatible; Biodegradable

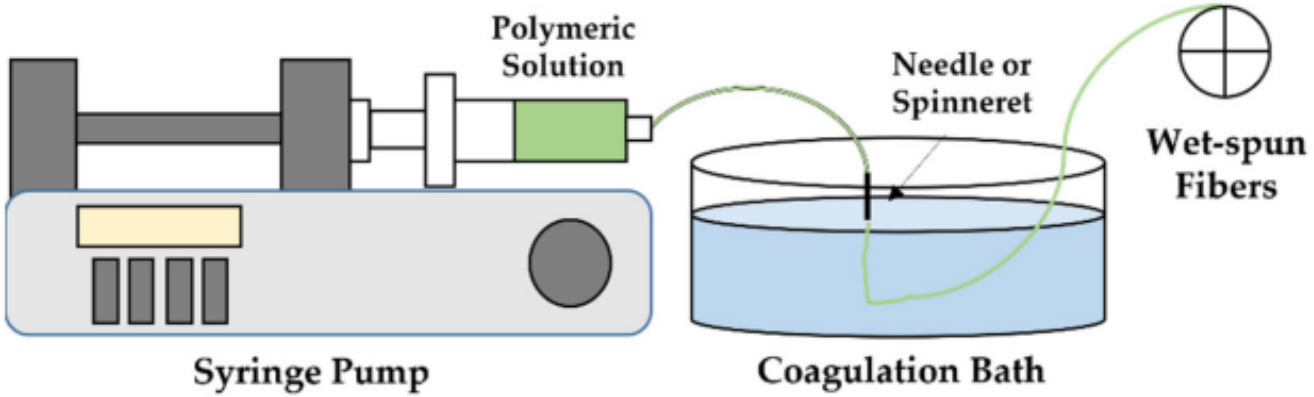
- Strong tissue adhesive properties;
- Accelerates tissue repair;
- Acts as a hemostatic agent.

- Non-toxic;
- Soluble at neutral to basic pH's;
- Antimicrobial activity.

Plotczyk, M. et al. Elsevier. 1 (2019)
Song, Q.; Zhang, Z.; Gao, J.; Ding, C. J. Appl. Polym. Sci. 2011, 119, 3282–3285

Wet-spinning

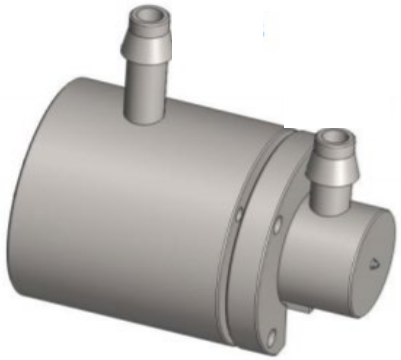
Technique based on a non-solvent-induced phase inversion process, in which a polymeric solution is extruded into a coagulation bath composed of a poor solvent or a non-solvent, giving rise to a continuous polymeric fiber, with a diameter of tens to hundreds of micrometers.



Miranda, C. *et al.* Pharmaceutics, 2022, 14. 164

Coaxial wet-spinning

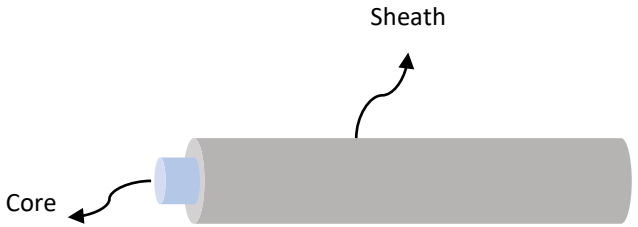
Coaxial spinneret



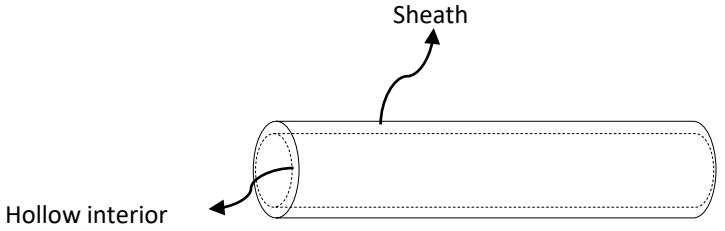
Coaxial fibers

Two polymer solutions are injected together into a co-axial spinneret and co-extruded towards a coagulation bath, generating a core-shell structure. In case of hollow fibers, air or coagulation bath is introduced through the innermost port.

Core-sheath fibers



Hollow fibers



Mirabedini, A. (2017). A thesis submitted in the fulfilment of the. 1–235.
Gao, L., Zhou, Y., Peng, J., Xu, C., Xu, Q., Xing, M., & Chang, J. (2019). *NPG Asia Materials*, *11*(1), 66.
McKeen, L. W. (2012). *Permeability Properties of Plastics and Elastomers*, 59–75.

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Goal

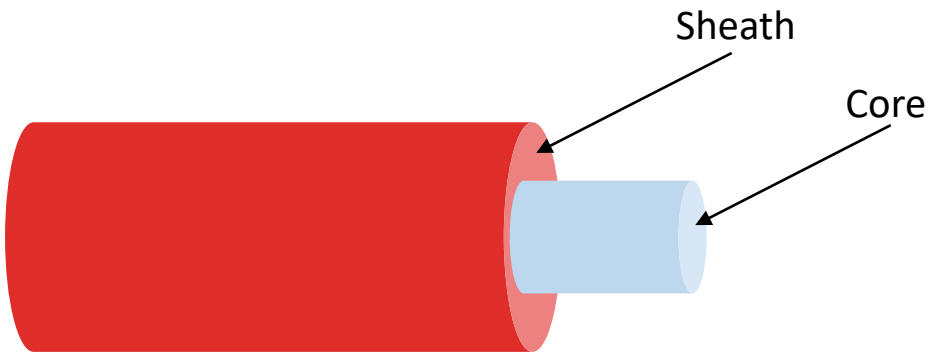
Engineer AAPV-loaded microfibers doped with NCMC via wet-spinning for human neutrophil elastase control and *Staphylococcus aureus* inhibition.

In chronic wounds, neutrophil elastase is 10-40% more abundant than in acute wounds. According to the World Health Organization, *S. aureus* is one of the most prevalent bacteria in chronic wounds.

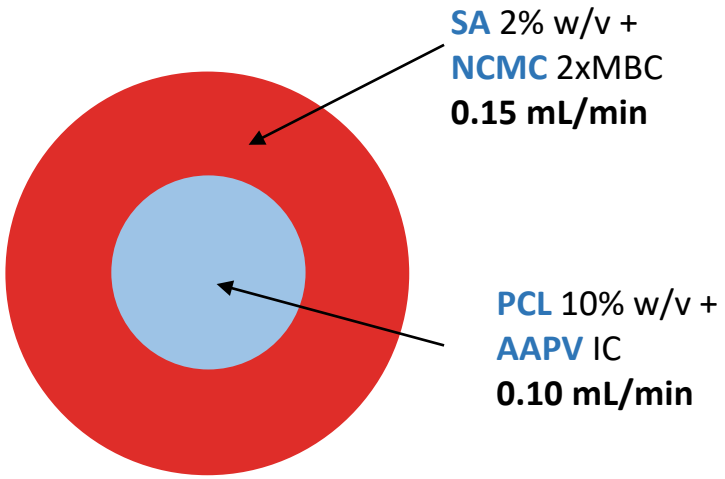
Felgueiras, H. et al. Colloids Surf B Biointerfaces 2017
https://www.who.int/

Coaxial wet-spun fibers

Side view



Cross-section view



Goal of Each Microfiber Component:
AAPV: regulate HNE enzymatic levels
SA: create and maintain a moist environment
PCL: maintain fibers' structural integrity
NCMC: antimicrobial activity

Coagulation bath: 2% w/v $CaCl_2$

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)

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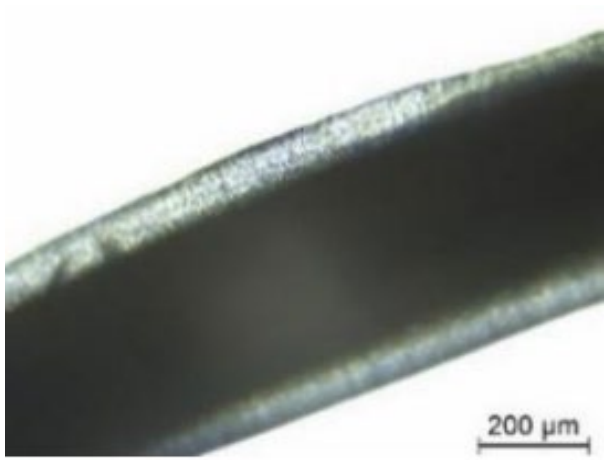
Fibers morphology

Macroscopic view

Microscopic view



Scanning Electron Microscopy (SEM)

Degree of swelling

Testing parameters:

- 10 mg fiber samples incubated in PBS at 37 ºC up to 24 h (measurements were conducted until mass equilibrium was reached)

$$DS\ (\%) = \frac{ws - wd}{wd} \times 100$$

ws – weight of swollen fibers
wd – weight of fibers prior to their incubation in PBS

Fiber	Degree of swelling (%)
SA	836.38 ± 115.60
SA-NCMC	1337.90 ± 86.82
PCL	49.20 ± 18.19
PCL-AAPV	54.25 ± 9.06
SA-PCL	172.82 ± 13.24
SA-PCL-AAPV	277.27 ± 5.96
SA-NCMC-PCL	283.40 ± 26.27
SA-NCMC-PCL-AAPV	324.36 ± 68.94

Hollow fibers presented highest degrees of swelling, due to SA's excellent hydration capacity; PCL-containing fibers were limited in their interactions with water molecules, due to PCL's hydrophobic nature.

Homem, N.C. *et al. Int. J. Mol. Sci.* **2021**

Degradation in PBS

Testing Parameters:

- 10 mg fiber samples incubated in PBS at 37 ºC up to 28 days;
- Samples were weighted after 1, 4, 7, 14, 21 and 28 days of incubation

$$mass\ loss\ (\%) = \frac{mi - mf}{mi} \times 100$$

mi – weight of samples at 0 h of incubation
mf – weight of samples after each incubation period

Fibers	Mass loss (%)					
	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
SA	1.30 ± 0.52	11.18 ± 3.99	74.37 ± 0.79	-	-	-
SA-NCMC	5.62 ± 2.79	12.23 ± 7.08	81.43 ± 1.97	-	-	-
PCL	-4.27 ± 11.36	-6.78 ± 2.26	-6.22 ± 7.19	-10.66 ±13.99	-13.94 ± 10.81	-30.67 ± 19.90
PCL-AAPV	-6.10 ± 28.12	-8.64 ± 3.01	-7.85 ± 15.75	-20.64 ± 1.33	-14.48 ± 2.06	-33.78 ± 12.58
SA-PCL	14.87 ± 5.85	12.42 ± 1.81	11.44 ± 4.55	16.41 ± 1.24	2.71 ± 1.05	3.46 ± 1.94
SA-PCL-AAPV	16.64 ± 9.01	16.65 ± 10.53	13.80 ± 12.34	21.59 ± 1.13	2.85 ± 1.77	6.11 ± 1.83
SA-NCMC-PCL	18.08 ± 3.69	20.96 ± 5.73	14.08 ± 3.24	22.91 ± 8.66	3.38 ± 2.87	6.07 ± 1.84
SA-NCMC-PCL-AAPV	20.37 ±10.56	23.16 ± 11.76	16.17 ± 14.35	25.34 ± 5.55	4.36 ± 3.42	6.84 ± 4.07

Fibrous structures were kept intact during 28 days of contact with PBS;
Hollow fibers were completely degraded after 14 days.

Mechanical behavior

Testing parameters:

- Housefield H5KS dynanometer associated with QMAT Materials Testing & Analysis software;
- Standard ASTM D5035;
- Speed: 25 mm/min;
- Load cell: 2.5-250 N;
- Filaments of 10 cm.

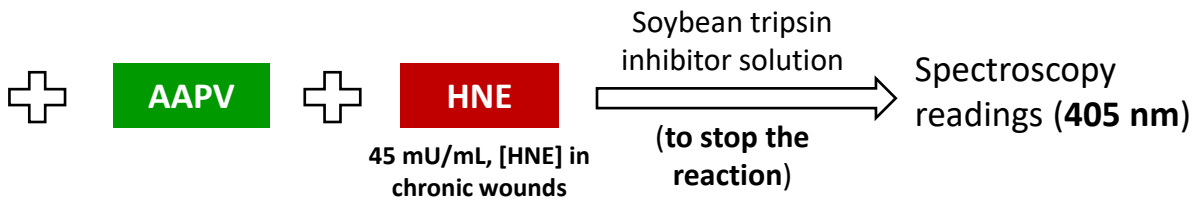
Fibers	Breaking strength (N)	Elongation at break (%)
PCL	0.02 ± 0.02	147.83 ± 22.56
PCL-AAPV	0.06 ± 0.04	133.91 ± 23.43
SA-PCL	0.04 ± 0.04	219.57 ± 60.39
SA-PCL-AAPV	0.06 ± 0.04	192.65 ± 54.47
SA-NCMC-PCL	0.04 ± 0.04	163.55 ± 62.14
SA-NCMC-PCL-AAPV	0.06 ± 0.04	156.76 ± 27.33

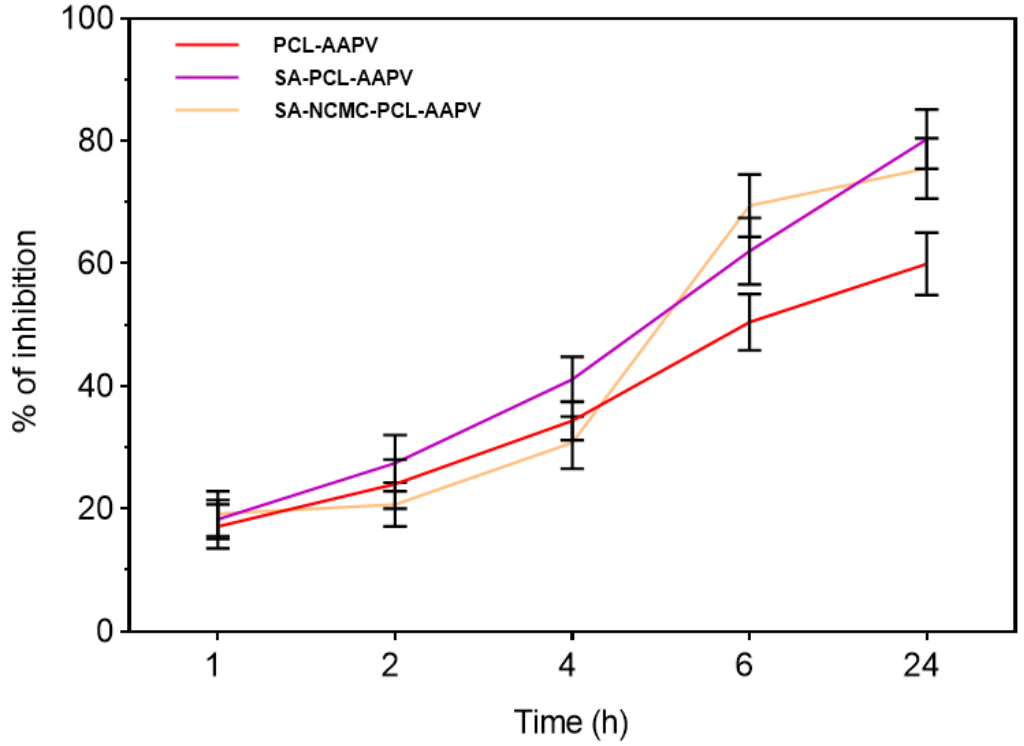
All fibers presented high maximum elongations, due to the elastic behavior of PCL;
Coaxial fibers presented higher elongations in comparison with monolayer fibers because of the shell protective effect, which delayed fiber rupture;
SA-PCL coaxial fibers presented the highest maximum elongations due to the absence of NCMC and AAPV, which interrupted the SA and PCL polymeric chains, respectively.

Rani, G. U. et al. *Reference Module in Materials Science and Materials Engineering* **2017**

Inhibition of HNE activity

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





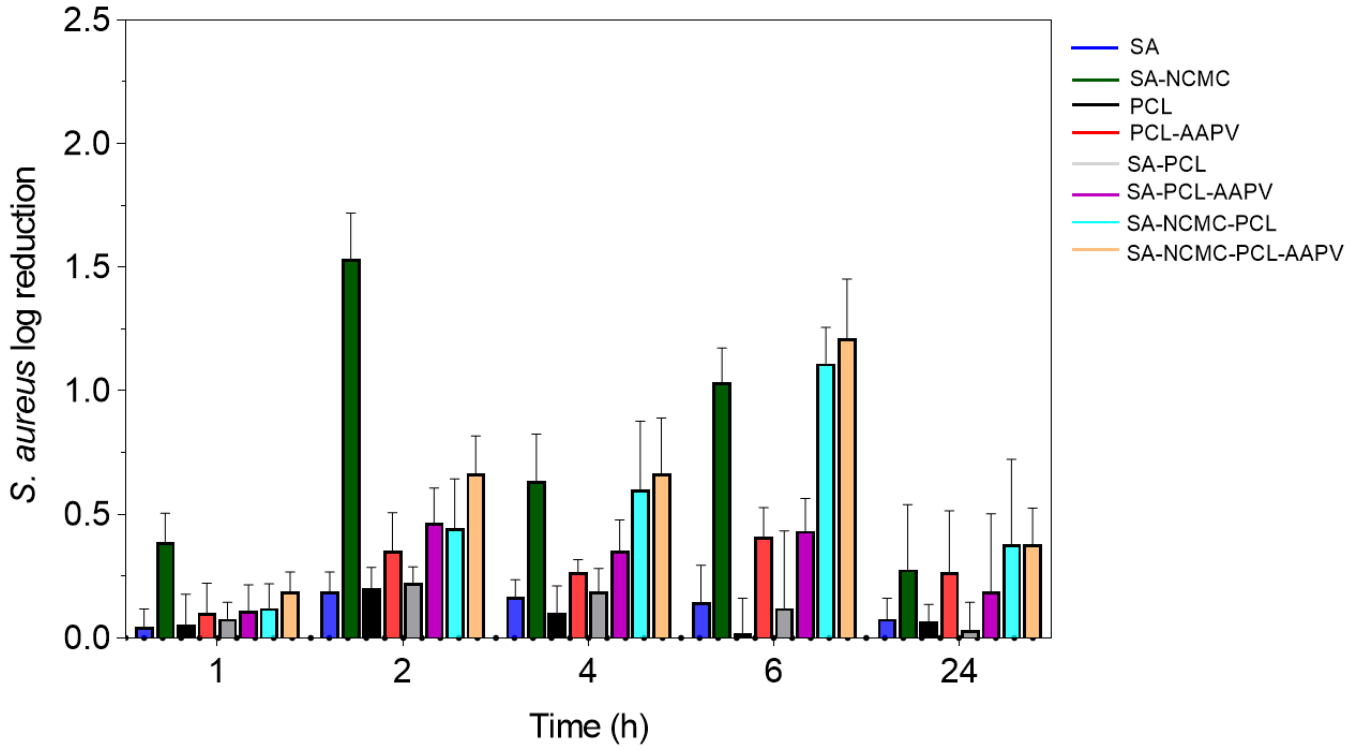
SA-NCMC-PCL-AAPV fibers presented the highest HNE inhibition at the 6 h mark.

Santos, F.C., et al. *Amino Acids* **2017**, *49*, 1077–108; Castro, V.I.B., et al. Tetrahedron 2016, 72, 1024–1030.

Antimicrobial activity

Testing Parameters:

- 1 × 10^5 CFUs/mL *S. aureus* suspension in Mueller Hinton Broth
- Samples of 10 mg immersed in 1 mL of bacteria suspension and incubated at 37 ºC and 120 rpm, for 1, 2, 4, 6 and 24 h;
- At each time point, bacteria suspensions were serially diluted in PBS and incubated at 37 ºC for 24 h;
- Grown colonies were counted, and results were expressed in log reduction.



NCMC-loaded fibers inhibited the most bacteria growth.
S. aureus activity was reduced by 90-99% at the 6 h mark.

Homem, N.C. *et al. Int. J. Mol. Sci.* **2021**

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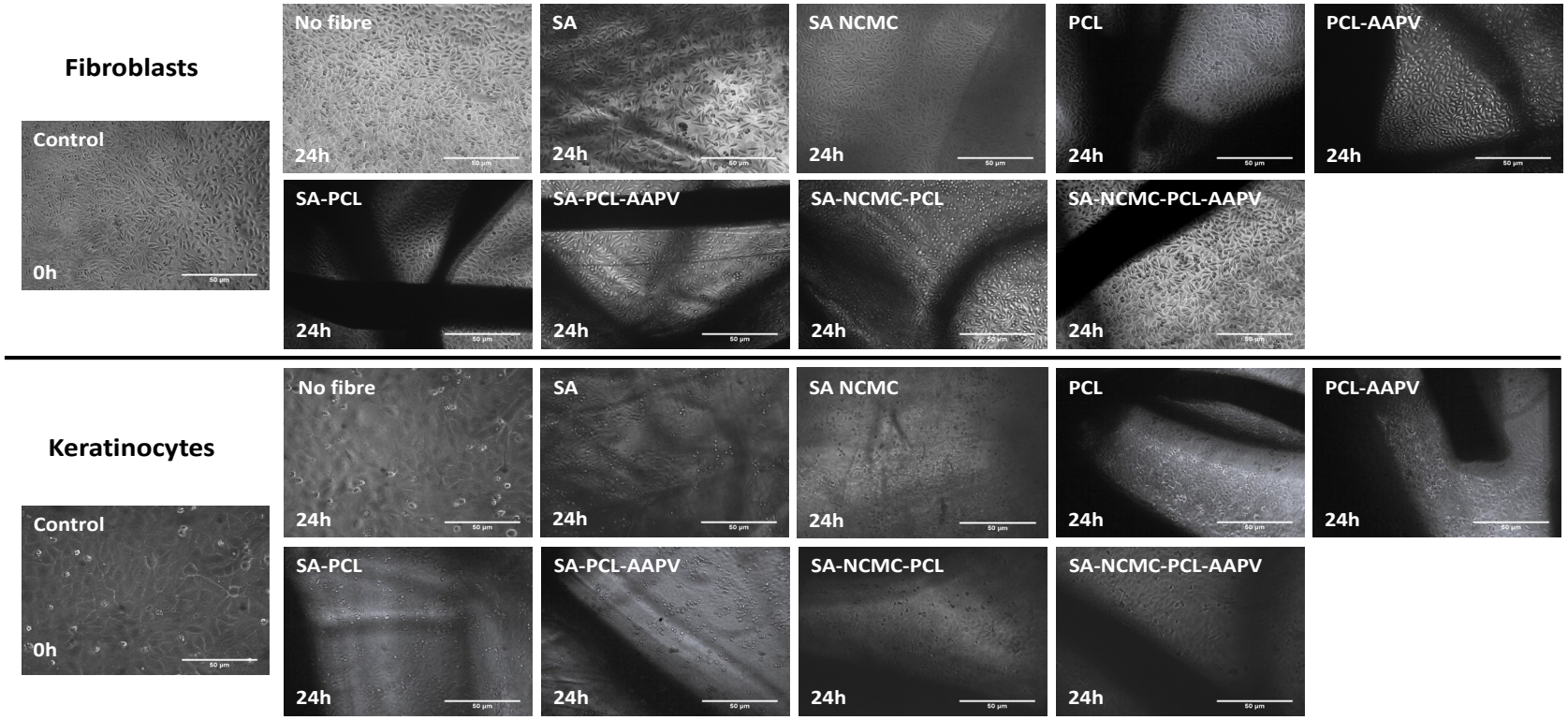
Cytocompatibility

- All coaxial wet-spun fibers were put in contact with mouse fibroblast cell lines (L929) and human keratinocytes cell lines (HaCaT) for 24h



Both cell lines maintained their viable and well-defined morphologies after contact with all wet-spun fibers.

Conclusions

The potential of the engineered coaxial fibers to serve as controlled release platforms for AAPV was demonstrated, along its inhibitory effect against HNE, particularly after 6 h of incubation.

The NCMC antibacterial activity against *S. aureus* was also established.

Cytocompatibility evaluations confirmed the safety of the engineered fibers.

Data confirmed the potential of this system to function as a multi-action delivery platform, suitable for prospective wound healing applications.

Coaxial wet-spun fibers loaded with enzyme-inhibiting peptide for chronic wound care

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