FABRICATION OF PEO ELECTROSPUN NANOFIBERS LOADED WITH PISCIDIN FOR ANTIBACTERIAL APPLICATIONS

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INTRODUCTION

The implementation of peptides as therapeutic agents presents some drawbacks. Remarkably, their poor stability hinders their supply and application in biological systems since their amide bonds can be easily proteolyzed by enzymes or hydrolyzed, which leads to short half-lives and their fast removal.

AIM

In this study, polyethylene oxide (PEO)/bovine serum albumin (BSA) and PEO/lysozyme (LYS) electrospun nanofibers (NFs) were loaded with 0.1 % (w/w) Piscidin 1 (PIS).

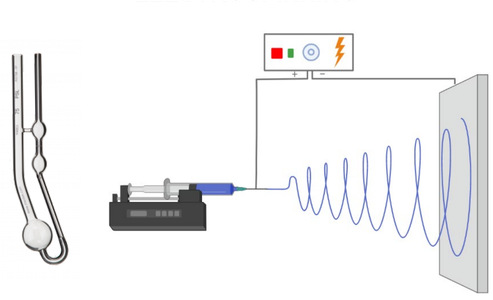
MATERIALS AND METHODS

SOLUTION PREPARATION

5-20 % LYS (w/w) + 3 % PEO (w/w) in MiliQ water

5-20 % BSA (w/w) + 3 % PEO (w/w) in MiliQ water

VISCOSITY DETERMINATION AND ELECTROSPINNING



Viscosity was determined to select the optimal solutions for electrospinning. **Parameters.** Feed rate: 0,25 mL/h. Voltage: 17 kV. Collector distance: 18 cm.

CHARACTERIZATION

Electron microscopy

ZEISS FESEM Sigma 300 VP

FTIR

Spectrometer FT-IR Bruker IFS 66/S

Antibacterial activity

Minimum inhibitory concentration (MIC) and mínimum bactericidal concentration (MBC) against *A. salmonicida, E. coli*, *B. circulans*, *B. subtilis, M. luteus, S. epidermidis*

PISCIDIN ADDITION AND CHARACTERIZATION

The MIC of PIS was first determined against six bacterial species. The solutions of 15% protein and 3% PEO were chosen to add 0.1% (w/w) of PIS since they formed NFs with an adequate appearance and diameter. The synthesized NFs were subsequently characterized.

RESULTS AND DISCUSSION

1. Viscosity

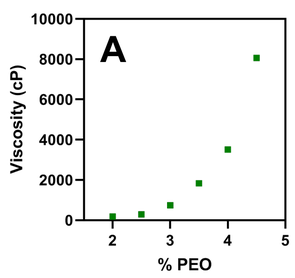


Fig 1. Viscosity in cP of: **A**. PEO, **B**. BSA and **C**. LYS solutions. The viscosity of the PEO solutions was measured to determine the electrospinning range according to the literature. Finally, 3% (w/w) of PEO was chosen to make the solutions with proteins. The viscosity of the BSA and LYS solutions + 3% PEO increased with the protein content.

2. Morphology and diameter of nanofibers

BSA-PEO NFs | **LYS-PEO NFs** | **BSA-PEO-PIS NFs** | **LYS-PEO-PIS NFs**

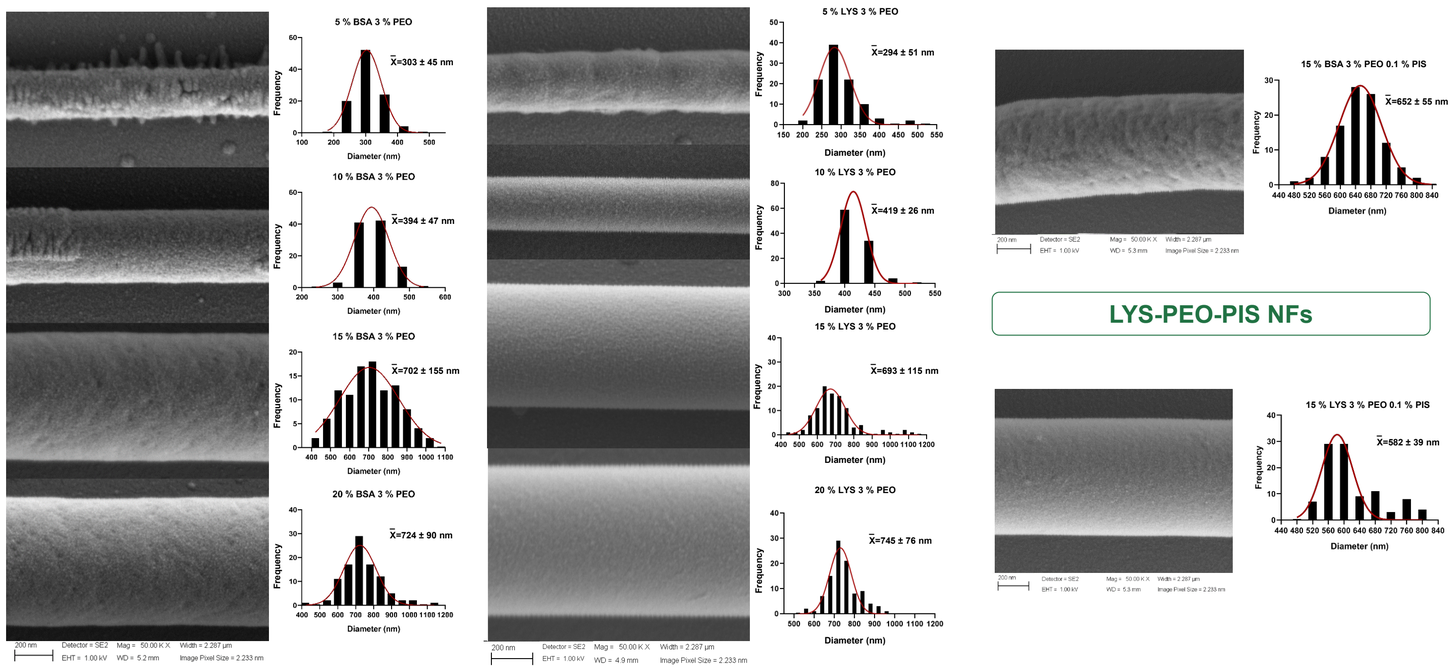


Fig 2. Electronic microscopy images and diameter frequency histograms of BSA-PEO, LYS-PEO, BSA-PIS-PEO and LYS-PEO-PIS NFs. In general, the diameter of the NFs increased with the amount of protein (BSA or LYS). In addition, the appearance of the NFs was smoother with a greater amount of protein. BSA NFs showed a more porous appearance than those of LYS. NFs of the 15% protein solutions were chosen to add piscidin due to their uniform appearance and adequate diameter.

3. FTIR

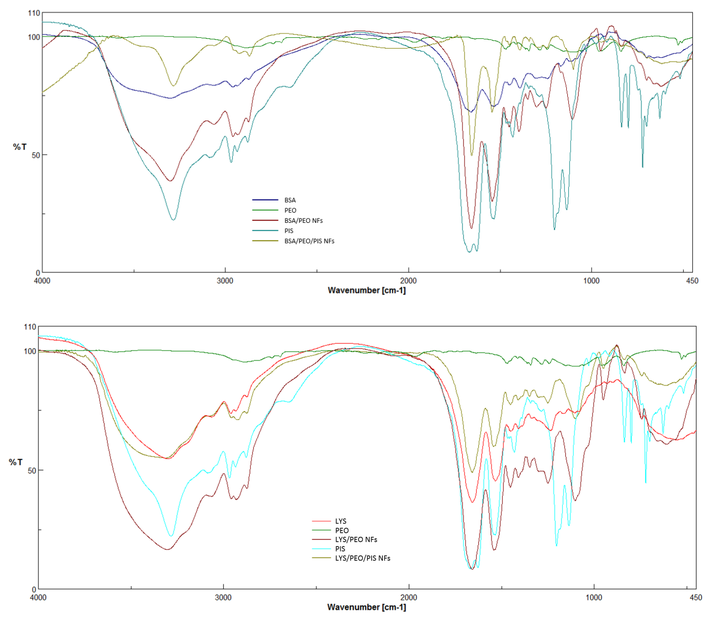


Fig. 3. Infrared spectra of BSA/PEO/PIS (top) and LYS/PEO/PIS (bottom) nanofibers. Characteristic bands of the proteins (amide A, I, II and III, 3305, 1658, 1533 and 1237 cm^{-1}, respectively) and polymer (CH_2 asymmetric rocking, 956 and 840 cm^{-1}) were also present in the BSA/LYS-PEO and BSA/LYS-PEO-PIS NFs samples. No differences were observed in the spectrum of the BSA/LYS-PEO and BSA/LYS-PEO-PIS samples.

4. Antimicrobial activity

Table 1. MIC and MBC (µg/ml) of LYS and LYS NFs against *B. circulans*. Screening was first performed by plate diffusion against all bacteria. *B. circulans* was the only bacterial species inhibited.

LYS		5 % LYS		10 % LYS		15 % LYS		20 % LYS	
MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBS
7.5	12.5	10.0	17.5	7.5	15.0	5.0	12.5	5.0	12.5

Table 2. MIC and MBC (µg/ml) of PIS against all bacteria. Screening was first performed by plate diffusion and all bacterial species were inhibited.

A. salmonicida		*B. circulans*		*B. subtilis*		*E. coli*		*M. luteus*		*S. epidermidis*	
MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBS	MIC	MBC
7.8	15.6	15.6	15.6	15.6	31.3	31.3	62.5	15.6	31.2	3.9	7.8

Table 3. MIC and MBC (mg/ml) of BSA-PEO-PIS and LYS-PEO-PIS NFs against all bacteria. In general, the MICs and MBCs of LYS-PEO-PIS NFs were lower than those of BSA-PEO-PIS, even in bacteria not previously inhibited by LYS.

	BSA-PEO-PIS		LYS-PEO-PIS	
	MIC	MBC	MIC	MBC
A. salmonicida	5.5	44	1.5	2.8
B. circulans	11	44	0.005	0.0078
B. subtilis	5.5	11	1.5	5.5
E. coli	12	24	5.5	22
M. luteus	1.5	22	2.8	11
S. epidermidis	0.34	0.68	0.34	0.68

CONCLUSIONS

In the present study NFs were synthesized by electrospinning incorporating proteins (BSA/LYS) and a peptide (PIS). FESEM results showed that the NFs with high protein content show a uniform appearance and that the addition of more protein increases the diameter of the NFs. In addition, FTIR studies showed that the protein structure is maintained in the NFs. NFs with PIS were able to inhibit all bacteria tested and their antimicrobial capacity was higher in combination with LYS.

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