HIGH PROTEIN CONTENT NANOFIBERS AS NOVEL PLATFORMS FOR ANTIBODY AND ANTIMICROBIAL PEPTIDE LOADING

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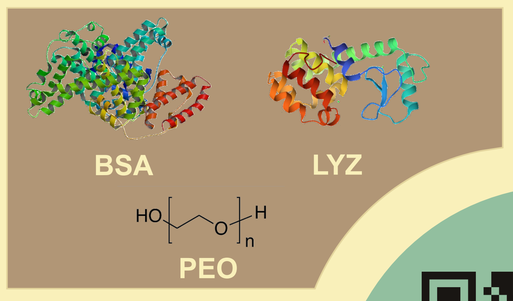
Introduction

The desirable characteristics of **nanomaterials**, such as **nanofibers** (NFs), make them suitable for implementation in biomedical applications. Concerning the material used for NF synthesis, **proteins** may offer advantages, as they present properties such as biocompatibility, biodegradability, or intrinsic functions. However, it can pose a challenge due to the possibility of their degradation or denaturation. For this reason, it is often necessary to blend with other natural or synthetic polymers to enhance these properties.

The integration of bioactive compounds into protein NFs can provide additional benefits, enabling biological functions with potential for various applications. Therefore, this study aimed to fabricate electrospun NFs with high content of one of two model proteins (**BSA** or **LYZ**) using polyethylene oxide (**PEO**) as copolymer. After optimization, small quantities of the antimicrobial peptide (AMP) **piscidin** (**PIS**) or the antibody (Ab) horseradish peroxidase (**HRP**)-conjugated Anti-Mouse **IgG** were added as a proof of concept.

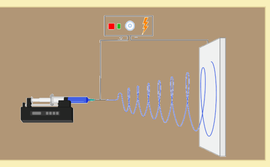


Materials and methods

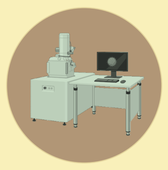
First, 3% (w/w) solutions of PEO and 5-20% (w/w) protein (BSA or LYZ) were prepared. The viscosity of the solutions was determined using Cannon-Fenske viscometers.

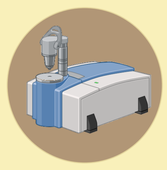
NFs were synthesyzed through electrospinning.

Electrospinning parameters
Voltage: **17 kV**
Tip-to-collector distance: **18 cm**
Flow rate: **0.25 mL/h**

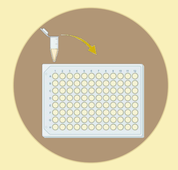


The 3% PEO and 15% protein solutions were selected to add 0.1% of either PIS or HRP-IgG, as they formed NFs with suitable size and appearance. The NFs were characterized through:


Electron microscopy


Infrared spectroscopy


Electrophoresis


Cytotoxicity


Activity assays

Results - Characterization

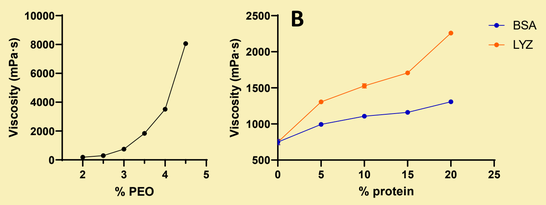


Figure 1. Viscosity (mPa·s) of solutions with PEO (3%) and different protein concentrations. The viscosity of the solutions increased with the protein concentration.

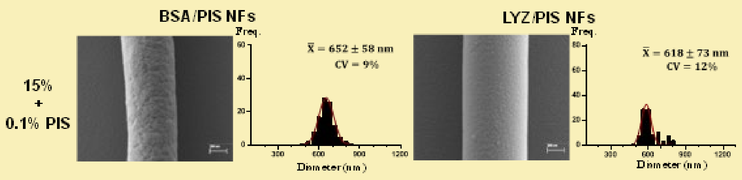


Figure 2. Electron microscopy images and frequency histograms of the diameters of BSA-PIS and LYZ-PEO NFs. The BSA NFs exhibited a more porous appearance and a larger size (652 nm) compared to the LYZ-PIS NFs (618 nm).

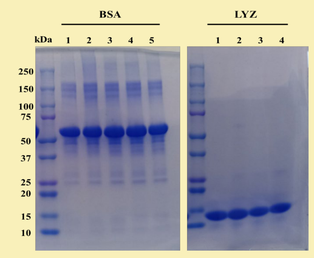


Figure 3. SDS-PAGE analysis of BSA and LYZ NFs. In both cases, lane 1 corresponds to the control, and the consecutive lanes correspond to increasing protein concentrations. No degradation was observed in any case.

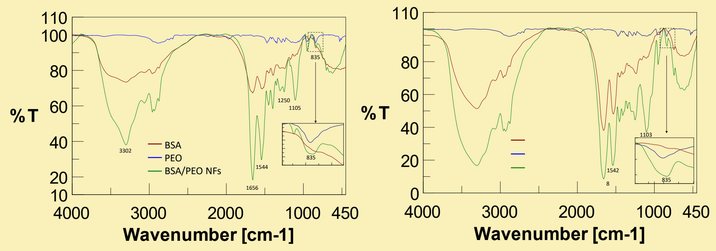


Figure 4. Infrared spectrum of BSA (left) and LYZ (right) NFs. The characteristic bands of the proteins (amide A, I, II, and III, at 3305, 1658, 1533, and 1237 cm^{-1}, respectively) and the polymer (asymmetric CH_2 rocking, at 956 and 840 cm^{-1}) were also found in the NFs.

Results – Biological Properties

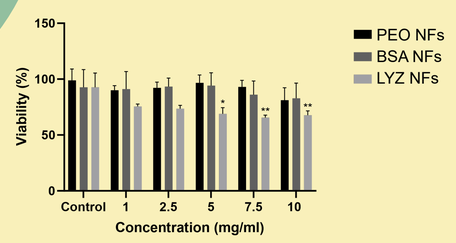


Figure 5. Viability (%) of HaCaT cells exposed to increasing concentrations of PEO, BSA and LYZ NFs determined by MTT assay after 24 h of incubation. Statistical differences between groups were calculated using a two-way ANOVA with Tukey's multiple comparisons correction method. Only LYZ NFs showed cytotoxicity at concentrations higher than 5 mg/mL.

Bacteria	MIC (μg/mL)			MBC (μg/mL)		
	PIS	BSA/PIS NFs	LYZ/PIS NFs	PIS	BSA/PIS NFs	LYZ/PIS NFs
A. salmonicida	7.8	31.3	7.8	15.6	249.6	15.6
B. circulans	15.6	62.6	5.0	15.6	249.6	7.8
B. subtilis	15.6	31.3	7.8	31.3	62.6	31.3
E. coli	31.3	62.6	31.3	62.6	124.8	124.8
M. luteus	15.6	15.6	7.8	31.3	124.8	62.6
S. aureus	31.3	31.3	31.3	62.6	62.6	62.6
MRSA	15.6	15.6	7.8	31.3	31.3	31.3
S. epidermidis	3.9	3.9	2.0	7.8	7.8	2.0

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of commercial PIS and the proportion of PIS contained in the BSA/PIS and LYZ/PIS NFs against *A. salmonicida, B. circulans, B. subtilis, E. coli, M. luteus, S. aureus, MRSA and S. epidermidis.*

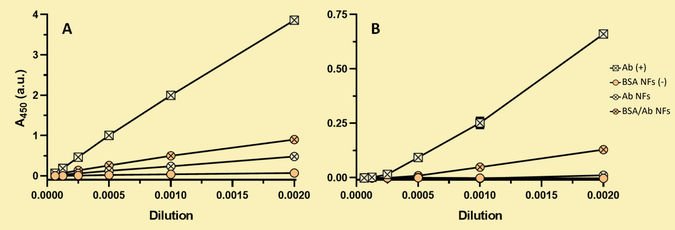


Figure 7. Peroxidase and antigen-recognition activity of NF-encapsulated HRP-IgG. Two-fold serial dilutions starting in 1:500 (i.e., 0.002 dilution) of experimental and control samples were tested for enzymatic peroxidase (A) and antigen-recognition (B) activity. Represented sample groups include HRP-IgG as positive control (Ab (+)), and BSA/PEO (negative control, BSA NF (-)), HRP-IgG-loaded PEO (Ab NF), and HRP-IgG-loaded BSA/PEO (BSA-Ab NF) NFs.

Conclusions and future perspectives

- ✓ NFs with high amounts of protein (BSA or LYZ) were successfully synthesized as stable encapsulation platforms.
- ✓ The appearance and distribution of the proteins in the NFs, as well as their structural integrity, which remained intact even at higher concentrations, indicate the potential of the synthesized NFs for stable and effective biomedical applications.
- ✓ No toxicity of NFs was found up to 5 mg/mL in human skin keratinocytes (HaCaT).
- ✓ PIS NFs were effective against different pathogenic bacteria, suggesting its broad-spectrum activity.
- ✓ The Ab activity was preserved in the NFs incorporating HRP-IgG, with preservation being notably higher in the NFs containing BSA.

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