

Assessment of Linear and Cyclic Peptides' Immobilization onto Cross-Linked, Poly(vinyl alcohol)/Cellulose Nanocrystal Nanofibers Electrospun over Quartz Crystal Microbalances with Dissipation Sensors

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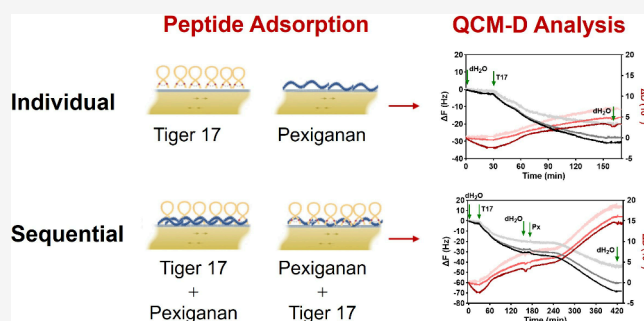


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ABSTRACT: Immobilization of peptides onto nanofiber dressings holds significant potential for chronic wound treatment. However, it is necessary to understand the adsorptive capacity of the produced substrates and the binding affinity of the peptides to determine the interface success. This study aims at exploring for the first time the influence of electrospun poly(vinyl alcohol)-based nanofibers on the adsorption of a cyclic peptide, Tiger 17, and of a linear peptide, Pexiganan, using quartz crystal microbalance with dissipation monitoring (QCM-D). PVA fibers reinforced with 0, 10, and 20% w/v cellulose nanocrystals (CNC) were electrospun directly onto QCM-D sensors and, posteriorly, cross-linked by glutaraldehyde vapor. Adsorption levels of Pexiganan were the highest ($\sim 7348 \text{ ng/cm}^2$) on C80/20 PVA/CNC electrospun fibers, while the time to achieve equilibrium was the longest ($\sim 235 \text{ min}$). In contrast, the adsorption mass with cyclic Tiger 17 was the highest ($\sim 3428 \text{ ng/cm}^2$) on C100/0 PVA, reaching equilibrium after nearly 123 min. In sequential deposition, the combination Tiger 17 + Pexiganan on C100/0 fibers attained the highest number of bound peptide molecules, with $\sim 55\%$ of Tiger 17 and $\sim 45\%$ of Pexiganan. Elastic shear modulus data on this peptide sequence, over the C80/20 electrospun mats, reported 220 and 249 kPa for each peptide, respectively, indicating the formation of stable bonds with the surface. The results contributed to the understanding of the immobilization of linear and cyclic peptides, never studied in combination, and their mutual influence on polymeric substrates for engineering potential wound treatment strategies.



INTRODUCTION

In the past few years, electrospinning has been gaining importance due to its versatility and simplicity in producing nanofibrous structures. This technique has the potential of producing a wide range of membranes for being used on filtration, electrochemical components, and biomedical purposes as the wound dressings.^{1,2} The high specific surface area and porous nature of electrospun nanofibrous mats that mimic the structural organization of the extracellular matrix (ECM) have generated opportunities for developing systems for the targeted and controlled delivery of antibacterial drugs and other therapeutic agents.^{3,4}

Electrospun nanostructures are predominantly produced from synthetic polymers because of their easy accessibility and processing; however, there are limitations such as their hydrophobicity and absence of cell recognition sites.⁵ Poly(vinyl alcohol) (PVA) is a synthetic, nontoxic polymer commonly used in biomedicine because of its hydrophilicity, large swelling capacity, biodegradability, high antifouling capability, chemical stability, high film-forming ability, and

good mechanical properties.^{6,7} Despite their advantages, PVA is known to reduce cell adhesion and to alter cells' morphology.⁸ To mitigate these issues, natural renewable cellulose-based nanoscale fillers, such as cellulose nanocrystals (CNC), have been combined with PVA. Various studies have demonstrated CNCs' capacity for promoting cell attachment and subsequent proliferation and migration because of their excellent water affinity and large surface area (multiple binding sites).⁹ In addition, CNCs have been recognized for their enhanced mechanical strength, conferring exceptional mechanical properties to high-performance nanocomposites.¹⁰

The functionalization of these nanofibrous nanocomposites with antimicrobial and immunomodulatory peptides can

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Table 1. Molecular Weight of Pexiganan and Tiger 17 and Hydrophobicity and Respective pK_a of the α -Carboxyl Group (pK_{a_1}), α -Ammonium Ion (pK_{a_2}), and Side Chain Group (pK_{a_3}) of Different Amino Acids That Compose These Peptides

Molecular weight (Da)	Pexiganan					Tiger 17						
	2581.1					1376.9						
	Amino acid Properties	Amino acid (letter code)	pK _{a₁}	pK _{a₂}	pK _{a₃}	Properties	Amino acid (letter code)	pK _{a₁}	pK _{a₂}	pK _{a₃}	Properties	
	Glycine (G)	2.34	9.60			Hydrophobic	Tryptophan (W)	2.46	9.41			Hydrophobic
	Isoleucine (I)	2.32	9.76			Hydrophobic	Cysteine (C)	1.92	10.70	8.37		Hydrophobic
	Lysine (K)	2.16	9.06	10.54		Hydrophilic	Lysine (K)	2.16	9.06	10.54		Hydrophilic
	Phenylalanine (F)	2.20	9.31			Hydrophobic	Proline (P)	1.95	10.64			Hydrophobic
	Leucine (L)	2.33	9.74			Hydrophobic	Arginine (R)	1.82	8.99	12.48		Hydrophilic
	Alanine (A)	2.35	9.87			Hydrophobic	Histidine (H)	1.80	9.33	6.04		Hydrophilic
	Valine (V)	2.29	9.74			Hydrophobic						

generate new infection management and immunomodulatory therapies, with proven effectiveness.¹¹ Antimicrobial and immunomodulatory peptides have emerged as effective alternatives to antibiotics. In contrast to conventional antibiotics, antimicrobial peptides (AMPs) penetrate and disrupt the bacterial cell membranes, reducing the likelihood of inducing resistance.¹² One of the most well explored AMPs is Pexiganan, a 22-amino acid synthetic analogue of the peptide magainin II that exhibits an α -helical structure, obtained from the skin of the African clawed frog *Xenopus laevis*.¹³ In clinical trials, Pexiganan reached phase III for the diabetic foot ulcer (DFU) handling but was not shown to be as efficient as ofloxacin, a Food and Drug Administration (FDA)-approved drug.¹⁴ Yet, Lipsky et al. demonstrated that bacterial resistance may emerge in patients receiving ofloxacin, which was not detected among patients receiving Pexiganan.¹⁵ Tiger 17, a 11-amino acid cyclic peptide, derived from tigerin precursors, has been deemed successful in instigating the different phases of diabetic ulcers' healing, namely, inflammation, proliferation, and remodeling.¹⁶ Recently, our team evaluated the Tiger 17 antimicrobial and blood clotting capacities, revealing important antibacterial effects against *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria and in accelerating the coagulation of blood plasma, respectively. The cytocompatibility of Tiger 17 has been corroborated by Tang et al.'s data, showing its excellent capability in promoting the proliferation of fibroblasts and keratinocytes.¹⁶ Despite their evident contribution to wound healing, the immobilization of Pexiganan and Tiger 17 onto nanostructured substrates, either individually or combined, may alter their performance. Thus, understanding the interactions established between these specific linear and cyclic peptides and polymeric surfaces at a molecular level is a novelty and is essential to comprehending their biological effects.¹⁷ Ellipsometry, surface plasmon resonance, sum frequency generation, atomic force microscopy, and quartz crystal microbalance with dissipation monitoring (QCM-D) have been some of the approaches used to analyze these interactions.¹⁸ In contrast to other techniques, QCM-D has the ability to provide complete information about the viscoelastic properties and mass of the adsorbed molecules but also of the hydrodynamically coupled solvent.^{19,20} Modifying the detection area of a QCM-D sensor with electrospun nanofibers has already been used to produce sensors capable of detecting ammonia levels, acetic anhydride vapor, and humidity in surrounding environments,^{21–23} to test the efficiency of cross-linking processes,²⁴ and to investigate the effects of polymeric nanofiber surfaces toward biofilm

formation,^{25,26} protein selectivity,^{27,28} and cell adhesion.²⁹ Here, PVA/CNC were examined at 100/0, 90/10, and 80/20% w/v ratios to assess the CNC influence on peptide adsorption. Nanofibers were directly produced by electrospinning over QCM-D sensors and were successfully cross-linked via glutaraldehyde (GA) vapor. To the best of our knowledge, this is the first report to describe PVA-based electrospun fibers deposited directly on top of QCM-D sensors and subsequently subjected to a GA vapor cross-linking process. Furthermore, another important novelty of this study focused on the analysis of the interactions established between these two structurally different peptides as well as their individual and combined adsorption interactions with these developed polymeric surfaces. This study together with other research already carried out by our team, in which binding of the same peptides was observed on spin-coated surfaces,³⁰ contributes to a much deeper understanding of the influence of complex nanoporous surfaces on the adsorption profile of structurally different peptides. Thereby, this research aimed at assessing the influence of the surface properties on the adsorption of structurally different peptides, namely, on their structural arrangements as well as adsorption restrictions one peptide imposes on another for developing an effective wound dressing.

MATERIALS AND METHOD

Materials. PVA with a degree of hydrolysis of about 88% and a molecular weight of 78000 g mol⁻¹ was obtained from Polysciences, Inc. (USA), and CNC (diameters ~75 nm and polydispersity index ~0.181) was obtained from CelluForce (Canada). Glutaraldehyde (GA; 25% aqueous solution and purity ≥98%) and 11-amino-1-undecanethiol hydrochloride (AUT; purity ≥98%) were purchased from Sigma-Aldrich. The same supplier was used for acquiring the reagents necessary for sensor cleaning, namely, Milli-Q ultrapure water, dimethyl sulfoxide (DMSO, ≥99.5%), ammonium hydroxide (30%), and hydrogen peroxide (30%). Gold-QCM-D (QSX301; nominal resonance frequency 4.95 MHz ± 50 kHz, AT-cut, 14 mm diameter, and 0.3 mm thickness) was acquired from Biolin Scientific. Peptides, cysteine-modified Pexiganan (purity >95%) and Tiger 17 (purity >95%), were provided by Isca Biochemicals (U.K.). The molecular weight (Mw) of Pexiganan and Tiger 17 and the hydrophobicity and pK_a values of all amino acids that make up these peptides are present in Table 1. All of the reagents were used without further purification.

QCM-D Sensor Preparation. Prior to all tests, the gold QCM-D sensors were cleaned as follows. The sensors were initially immersed in DMSO for 1 h in an ultrasonic bath and were then held at 80 °C for 15 min in a 5:1:1 v/v/v mixture of Milli-Q ultrapure water, hydrogen peroxide (30% w/w), and ammonia (25% w/w). The

sensors were then rinsed with Milli-Q ultrapure water, dried with filtered nitrogen, and finally exposed for 20 min to UV/ozone (UVO-Cleaner model no. 42, Jelight). To ensure that the polymeric layer adheres to the sensor surface, drops of a 1 mM solution of AUT in ethanol were placed over the sensor surface and left overnight at 4 °C. Finally, unbound AUT molecules were rinsed off with Milli-Q ultrapure water.

QCM-D Sensor Coating. Electrospun nanofibers were deposited onto the AUT-modified QCM-D sensors to form a mat. The edges were carefully trimmed to remove any overhanging mat to avoid the loss of sensitivity during the measurements. The solutions for electrospinning were prepared by dissolving 10% (w/v) PVA/CNC at 100/0 (100% PVA), 90/10, and 80/20% (v/v) ratios, in distilled water (dH₂O) at 80 °C for 5 h. Afterward, to guarantee CNC dispersion, the solution was sonicated for 1 h. Polymeric solution viscosities and conductivities were measured at room temperature (RT) by means of a viscometer (Brookfield DV-II+ Pro using a 21 spindle) and conductivity meter (Zetasizer Nano ZS, Malvern Instruments), respectively.

During electrospinning, sensors were placed in the center of a planar collector placed 9 cm from an 18 gauge (G) steel capillary needle maintained at 20 kV for 3 min at a rate of 1.6 mL/h. These conditions were determined to be optimal for all polymer combinations without compromising the sensor sensitivity. RT and relative humidity (RH) were maintained at 20–22 °C and 40–50%, correspondingly. After deposition, sensors were dried overnight at 80 °C to remove residual solvents and environmental moisture. Considering which PVA is unstable in aqueous media, a cross-linking process with GA vapor was employed for 8 h at 80 °C. A 1 mL portion of GA per sensor was used (determined to be ideal for cross-linking without saturating the polymeric fibers). The entire process was carried out inside a vacuum-sealed desiccator. Finally, cross-linked PVA based sensors were dried overnight at 80 °C to eliminate residual traces of GA.

To facilitate sample identification, un-cross-linked electrospun fibers were labeled as 100/0, 90/10 and 80/20, while the cross-linked electrospun fibers were identified as C100/0, C90/10, and C80/20.

Surface Characterization. Scanning Electron Microscopy (SEM). SEM micrographs (Nova 200 NanoLab, SEM, FEI Company) of the electrospun sensors were obtained with an accelerating voltage of 10 kV. Images at 5,000 and 20,000× magnification were collected and processed by ImageJ software (version 1.44) for nanofiber diameter determinations before and after cross-linking.

Fourier-Transform Infrared Spectroscopy in Attenuated Total Reflectance Mode (ATR-FTIR). The polymers' blending and effectiveness of the cross-linking were analyzed via ATR-FTIR. Spectra were acquired using a Nicolet™ iS10 FTIR spectrometer (Thermo Fisher Scientific, USA) with a diamond ATR crystal cell. Each spectrum was obtained in transmittance mode from 45 scans at a resolution of 4 cm⁻¹ over 4000–550 cm⁻¹.

Contact Angle Measurements. An OCA 20 DataPhysics apparatus (Frankfurt, Germany), connected to the OCA15 plus software (version 1.2), was used to determine the water contact angle of the electrospun surfaces following the standard ASTM-D7334-08. Drops of 5 µL of dH₂O, with a dosage rate of 1 µL/s, were used to assess the wettability of the surfaces by the sessile drop method at 23 °C and RH of 50%. The evaluation of three electrospun sensors was performed on three randomly selected spots along the surface, with a total of nine measurements. Angle measurements were taken immediately after the drops' contact with the surfaces.

Peptide Surface Immobilization. Quartz Crystal Microbalance with Dissipation (QCM-D) Analyses. The shifts in frequency (ΔF) and dissipation (ΔD) that occur upon peptide adsorption to sensors coated with PVA/CNC electrospun mats were monitored by using a QCM-D instrument (Q-Sense E4 instrument, Biolin Scientific, Sweden). The coating thickness was found from the frequency data before and after the fiber's deposition. Experiments were conducted at RT. An initial baseline was obtained with peptides' solvent at a rate of 5 µL/min. Milli-Q ultrapure water passed over the electrospun QCM-D sensors before inserting any peptides.

Single-Peptide Adsorption Studies. Pexiganan and Tiger 17 were introduced individually into the QCM-D modules at a rate of 5 µL/min. Peptide solutions were prepared according to their minimum inhibitory concentrations (MICs).^{31,32} Because of their antimicrobial limitations, Pexiganan was prepared at 128 µg/mL (2 × MIC) and Tiger 17, at 40 µg/mL (double the concentration reported by Tang et al.³³ for inducing regenerative effects on CW). A constant flow was maintained until peptide saturation. Once this equilibrium condition was reached, Milli-Q ultrapure water again flowed over the sensors for 10 min at a rate of 5 µL/min to remove poorly bound peptide molecules. Tests were conducted on sensors with and without electrospun nanofibers (controls).

Sequential Peptide Adsorption Studies. PVA/CNC electrospun sensors were exposed to a sequence of two peptides as follows: (1) Pexiganan + Tiger 17 and (2) Tiger 17 + Pexiganan. Sensors without any coating were used as a control. Peptide solutions were prepared as explained in an earlier section. The adsorption of the first peptide was monitored until saturation, before introducing the second peptide into the modules, and left as well until a new adlayer reached equilibrium. After each equilibrium step, any poorly bound peptide molecules were removed by flowing Milli-Q ultrapure water for 10 min.

QCM-D Assessment Tools. The electrospun sensors registered ΔD shifts superior to 1×10^{-6} per 20 Hz drop in frequency during peptide adsorption. As result, the Voigt model, a viscoelastic modeling function commonly used to determine the total mass of the peptides and the coupled water molecules, was applied to calculate the adsorbed peptide masses (ng/cm²) and the elastic shear modulus (kPa).³⁴

To evaluate the peptides' adsorption behaviors, data from the 3rd to the 11th harmonics was acquired, but only the 3rd, 5th, and 7th harmonics were selected for comparison purposes. All parameters assessed (peptide adsorption mass, elastic shear modulus, and electrospun layer thicknesses) were determined using QTools software (Biolin Scientific). The elastic shear modulus values presented were calculated as an average of the equilibrium phase (saturation) of each peptide adsorbed onto the electrospun mats. The controls do not present any elastic shear modulus because their peptide adsorption was determined via the Sauerbrey equation. To determine the AUT layers and PVA/CNC mats' thicknesses over the sensors, stable baseline curves were first obtained with a clean QCM-D sensor. The AUT layer and the different PVA/CNC fibers were then deposited on these clean QCM-D sensors. The coated sensors were remounted precisely in the same locations in the module, and the frequency response was recorded again. The two frequency data files for each layer/fibrous mat, from the blank QCM-D sensors and coated sensors, were stitched using the "Stitch Data Files" function in QTools software. The Sauerbrey equation was used to determine Δm from which the layers thicknesses were calculated by dividing Δm , assuming polymeric densities of 0.9 g/cm³ and 1.2 g/cm³.

Statistical Analysis. The experiments were performed in triplicate. Numerical data are reported as the mean $\pm$ standard deviation (S.D.). Kruskal–Wallis and one-way analysis of variance (ANOVA GraphPad Prism 9.0 software) was used to determine the statistical significance. Multiple comparisons were made via Tukey's correction. Significance is defined as having $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of Solutions and Electrospun Fibers.

Prior to the deposition of electrospun fibers onto QCM-D sensors, an AUT coating was applied to enhance their adhesion to the sensors. The thickness of these AUT layers was determined at between $\sim 1.25 \pm 1.14$ and $\sim 5.60 \pm 1.25$ nm. AUT is a thiol molecule. The thiol group binds to the gold on the QCM-D sensor by strong metal–sulfur interactions (i.e., chemisorption), establishing close-packed self-assembled monolayers. This leaves the amine groups available for bonding at the surface.³⁵ These amine groups on AUT are

Table 2. Viscosity, Conductivity, and Zeta Potential of 100/0, 90/10, and 80/20 PVA/CNC Solutions and Wettability of the Cross-Linked Fibers

	Polymer ratios	Viscosity(cP)	Conductivity(μ S/cm)	Zeta potential(mV)	Contact angle(deg)
PVA/CNC	100/0	495.0 $\pm$ 4.8	718.0 $\pm$ 8.0	−0.1 $\pm$ 0.1	75.3 $\pm$ 12.2
	90/10	467.1 $\pm$ 10.0	724.0 $\pm$ 7.0	−1.6 $\pm$ 0.2	70.2 $\pm$ 15.1
	80/20	394.0 $\pm$ 10.1	880.0 $\pm$ 4.2	−2.3 $\pm$ 0.2	63.1 $\pm$ 18.1

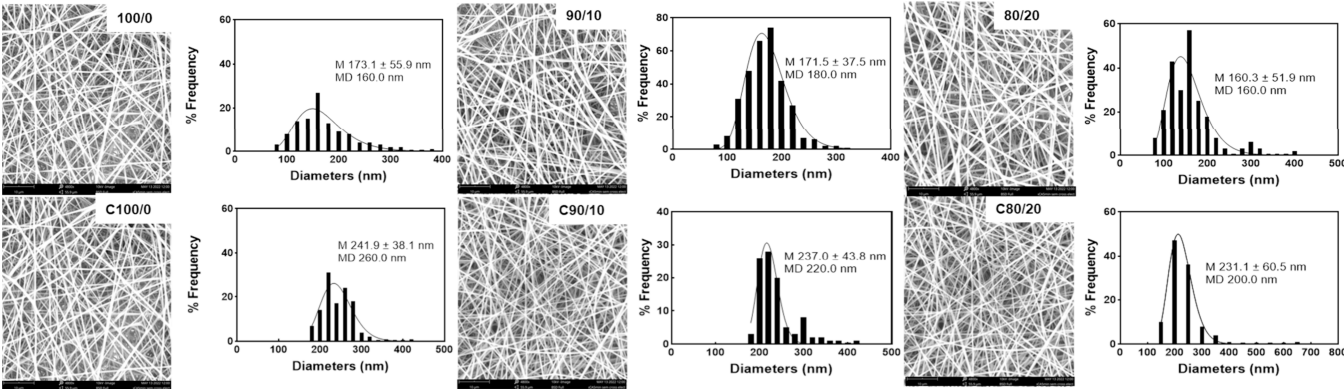


Figure 1. SEM micrographs of the electrospun PVA/CNC fibers before (100/0, 90/10, and 80/20) and after (C100/0, C90/10, and C80/20) cross-linking and respective diameter distribution histograms (with mean, M, and mode, MD, identified). Each image presents a magnification of 4800X, and scale bars represent 10 μ m. Statistical significance was found via Kruskal-Wallis's test by using multiple comparisons between un-cross-linked (100/0 vs 90/10 ns; 100/0 vs 80/20, * p < 0.0442; 90/10 vs 80/20, *** p < 0.0001) and cross-linked samples (100/0 vs 90/10 ns; 100/0 vs 80/20, ** p < 0.0037; 90/10 vs 80/20 ns) (ns, nonsignificant).

protonated under neutral media (experimental conditions), allowing electrostatic interactions between the positively charged AUT and the weakly negatively charged acetate groups of PVA and/or the sulfate groups from CNC (~12%).³⁶ Subsequently, the 100/0, 90/10, and 80/20% w/v PVA/CNC electrospun fibers were produced on top of these AUT-modified QCM-D sensors. As the viscosity, conductivity, and zeta potential of polymeric solutions are key factors influencing the diameter and morphology of electrospun nanofibers, measurements were conducted.³⁷ The addition of CNC to PVA reduced the solutions' viscosities and zeta potential but increased their conductivities (Table 2).

Consequently, the 100/0, 90/10, and 80/20 nanofibers' diameters decreased in the presence of CNC and their morphology was found to be defect-free (absence of beads), uniform, and continuous (Figure 1). This results in the reduction in the solutions' viscosity triggered by a decreased entanglement of the PVA chains, which also increases conductivity, induced by the CNC sulfate ester groups' negative charges.^{38,39} The presence of negative sulfate ester groups was confirmed by a decrease in the zeta potential as the CNC content was raised (Table 2). Solutions with low viscosities and high conductivities have been linked to small electrospun fiber diameters and the absence of beads since the fiber jets tend to withstand greater tensile forces.^{6,40} Various studies have also shown that the smallest fiber diameters have induced smaller pore sizes on electrospun mats. Therefore, 80/20 PVA/CNC electrospun mats could present the lowest pore sizes.^{41,42}

To prevent the instant solubility of the PVA-based fibers in an aqueous medium, chemical cross-linking via GA vapor was employed. Effective cross-linking was achieved by exposing the sensor surface to vapors produced by 1 mL of GA (per sensor) inside a vacuum-sealed desiccator held at 80 °C for 8 h. After this process, to remove residual GA traces, the cross-linked

sensors were left overnight at 80 °C. GA is a common chemical cross-linking agent used with great effectiveness to stabilize PVA scaffolds at an affordable cost and reduced or negligible cytotoxicity.⁴³ In the presence of GA, covalent bonds are formed between its aldehyde groups and the hydroxyl groups of PVA and CNC.⁴⁴ This cross-linking process increased the diameters of the nanofibers, resulting from the establishment of the acetal bonds between the GA and the polymers and from the swelling due to the water molecules present in the GA solution. After cross-linking, the electrospun nanofiber diameters increased ~40, 38, and 44% for C100/0, C90/10, and C80/20 PVA/CNC electrospun fibers, respectively (Figure 1). Similarly, an increase in the average mats' thickness was evident, going from ~157 nm for un-cross-linked fibers to ~237 nm for cross-linked fibers. Despite this diameter increase, the success of the process was confirmed by observing a stable baseline (without any ΔF and ΔD fluctuation) after the introduction of Milli-Q ultrapure water on the QCM-D modules (as seen in Figure S1.1 in the Support Information file) for all electrospun mats. This data showed the effectiveness of the process. All cross-linked electrospun mats showed uniform, continuous, and defect-free morphology (Figure 1). In fact, no detectable morphology differences were observed between un-cross-linked and cross-linked nanofibers or between polymer ratios.

Aside from the surfaces' morphology and topography, peptides' adsorption kinetics is also influenced by substrates' wettability.⁴⁵ Subsequently, the chemical composition, structural organization, roughness, and surface energy have been described as the main influencers of surface wettability.⁴⁶ The wettability of all fibers produced was measured via a sessile drop method by determining the contact angle formed among the dH₂O drop, the tested surface, and the surrounding atmosphere. All cross-linked electrospun fibers were shown to be hydrophilic. The contact angles decreased slightly as the

CNC content increased in the fibers (Table 1). Previous studies reported similar outcomes.^{47,48} With a higher CNC ratio in the formulations, the contact angle decreased. This results from the presence of many free $-OH$ groups on the CNC surface. Moderate wettability values, such as the ones obtained, can be beneficial, since superhydrophilic or hydrophobic surfaces have been considered to be less appropriate for inducing cell events required for successful wound healing.⁴⁸

By ATR-FTIR data, polymer blending and the cross-linking process were proven (Figure 2). All spectra revealed

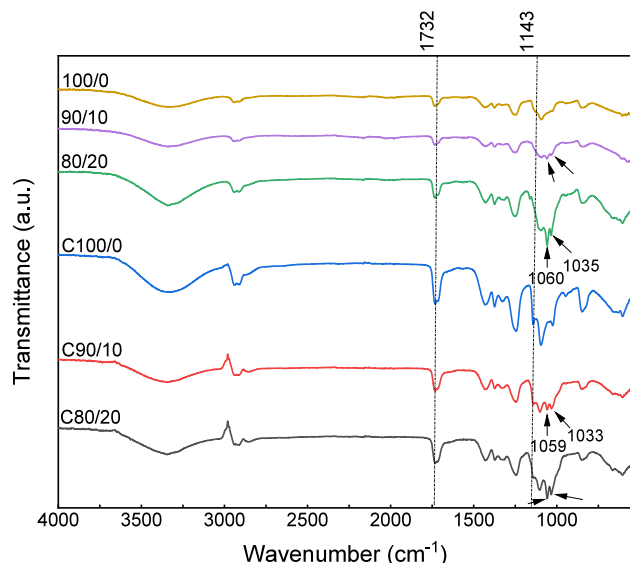


Figure 2. ATR-FTIR spectra of electrospun PVA/CNC fibers, before (100/0, 90/10 and 80/20) and after (C100/0, C90/10, and C80/20) cross-linking, were acquired between 4000 and 550 cm^{-1} . The arrows present in the spectra highlight the CNC characteristic peaks.

characteristic bands of PVA, with CNC-containing electrospun fibers showing evidence of this cellulosic reinforcement. In addition to the fiber's morphology, the wettability and

chemistry are also important features conditioning the type, amount, and conformation of peptides and proteins adsorbed onto a biomaterial's surface.⁴⁹

As expected, the spectra from un-cross-linked fibers were not as well defined as those of the cross-linked fibers (except for the 80/20 fibers) because of their reduced thickness and depth of the equipment readability ($0.7\text{--}2.0\ \mu\text{m}$). Regardless, all spectra showed the characteristic PVA bands (also confirmed via ATR-FTIR spectra of PVA powder in Figure S2.1): the $-O-H$ stretching vibration (at $\sim 3343\ \text{cm}^{-1}$), the $C-H$ stretching of the alkyl groups (~ 2942 and $2912\ \text{cm}^{-1}$), the $C=O$ stretching bond of acetate groups ($\sim 1732\ \text{cm}^{-1}$), the $C-H_2$ bending vibrations (~ 1428 and $\sim 1371\ \text{cm}^{-1}$), the $C-C$ skeletal vibration ($\sim 1248\ \text{cm}^{-1}$), the $C-C$ and $C-O$ groups' stretching vibrations ($\sim 1091\ \text{cm}^{-1}$), the rocking vibration of CH_2 ($\sim 943\ \text{cm}^{-1}$), and the $C-C$ stretching vibrations and the $O-H$ out-of-plane bending vibrations ($\sim 848\ \text{cm}^{-1}$).^{50,51} The presence of CNC on CNC-containing electrospun fibers was confirmed by the appearance of two characteristic bands related to the $C-OH$ stretching of CNC at $\sim 1060\ \text{cm}^{-1}$ (secondary $-OH$) and at $\sim 1035\ \text{cm}^{-1}$ (primary $-OH$) (highlighted in Figure 2 and Figure S2.1).⁵² The efficiency of the cross-linking process was proven by the emergence of two specific bands in the cross-linked fiber spectra, at $\sim 1143\ \text{cm}^{-1}$ associated with the establishment of acetal bonds ($-O-C-O-$ vibrations) between polymers and GA and an intensification of the band at $\sim 1732\ \text{cm}^{-1}$, evidencing the $C=O$ stretching bond of GA that remained in the fibers.⁵³ Considering that no new peaks were observed, it was possible that PVA and CNC did not suffer any chemical reaction but rather physical blending.

Single-Peptide Adsorption Studies. Tiger 17 ($40\ \mu\text{g}/\text{mL}$) and Pexiganan ($128\ \mu\text{g}/\text{mL}$) adsorption kinetics was monitored *in situ* on QCM-D sensors with (C100/0, C90/10, and C80/20 PVA/CNC) and without (control) a nanofibrous layer (Figure 3). On all electrospun fibers, ΔF drops and ΔD shifts were higher in Pexiganan, particularly on those containing CNC. Its extended linear architecture, chain size, and composition would have contributed to this result. Being a

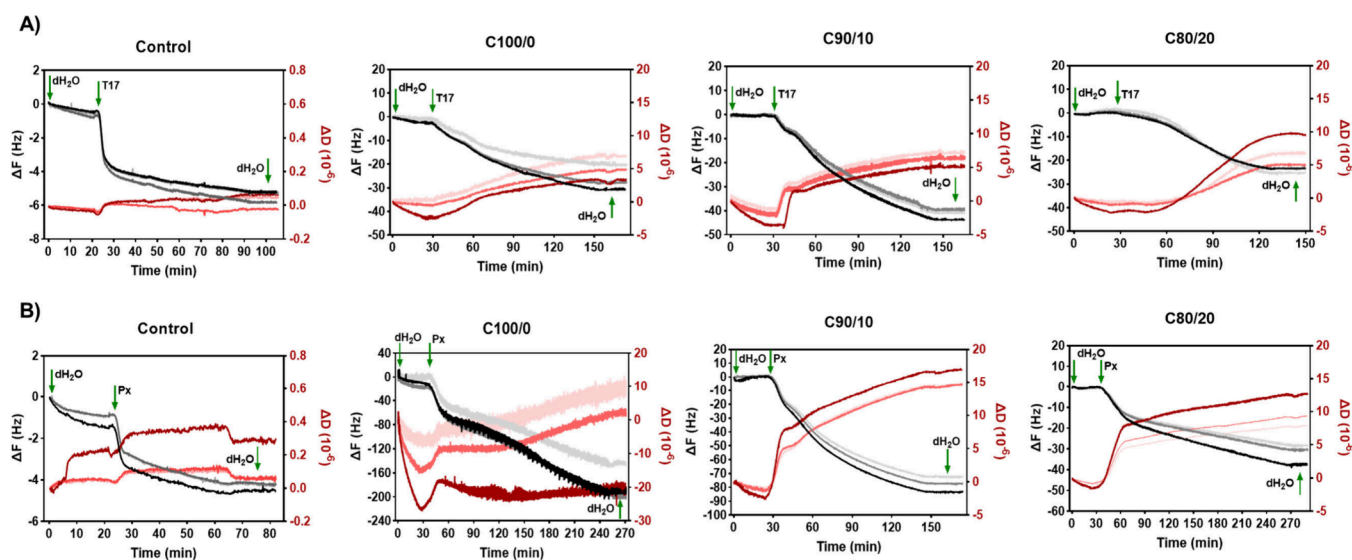


Figure 3. Frequency (ΔF) and dissipation (ΔD) plots of A) Tiger 17 (T17) and B) Pexiganan (Px) individual adsorption profiles on QCM-D sensors electrospun with C100/0, C90/10, and C80/20 PVA/CNC and without any coating (control). 3rd, 5th, and 7th overtones are shown; the higher the overtone, the lighter the colors.

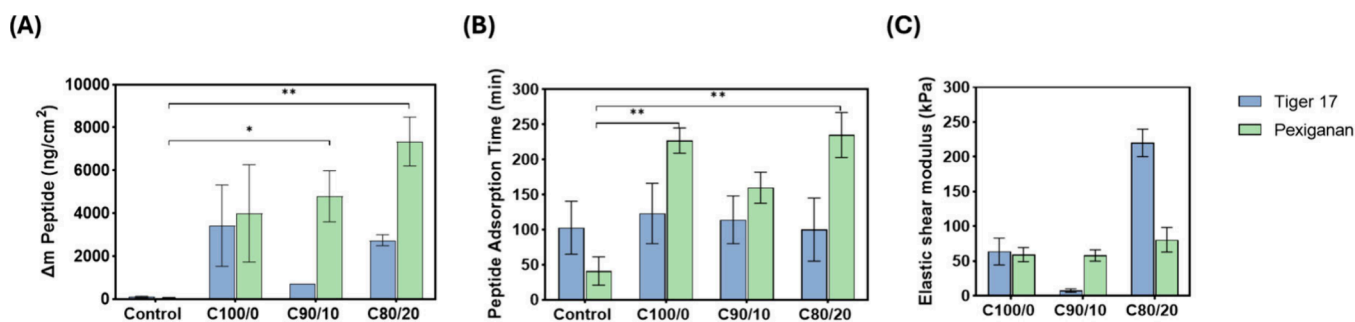


Figure 4. (A) Tiger 17 and Pexiganan adsorbed mass on control sensors (without any polymeric fibers) and on C100/0, C90/10, and C80/20 PVA/CNC electrospun sensors. (B) Time needed for Tiger 17 and Pexiganan to achieve surface saturation (equilibrium). Experiments were performed at a flow rate of 5 $\mu\text{L}/\text{min}$. (C) Elastic shear moduli (kPa) of Tiger 17 and Pexiganan at their adsorption equilibrium state (saturation). Statistical significance for (A), (B), and (C) was found by Kruskal-Wallis's test applying Dunn's test for multiple comparisons, which were indicated by * ($p \leq 0.05$, $**p \leq 0.01$).

linear peptide of 22 amino acids with a high degree of conformational freedom, Pexiganan would benefit from its multisite binding capacity to link to the large surface area provided by the electrospun nanofibers. Pexiganan's smaller elastic shear modulus also confirms these properties, in opposition to the superior elastic shear modulus shown by Tiger 17, evidencing its rigid and cyclic structure.⁵⁴ The presence of CNC on electrospun fibers enhanced the peptides' adsorption by means of strong, negatively charged sulfate groups in physiological media ($\text{pK}_a \sim 2.4$). These favored the electrostatic interactions with the peptides since, under physiological conditions, the amino acids present on Pexiganan and the Tiger 17 chain, namely, lysine and arginine, are positively charged (elevated pK_a (Table 1)).^{55,56} This enhanced peptides' adsorption onto CNC-containing mats, as also highlighted by the elastic shear modulus. These values were superior for both peptides on the C80/20 electrospun mats compared to those of other mats studied. It could indicate that stronger and more stable interactions were established between peptides and these CNC-containing surfaces.⁵⁷ On fibers composed only of PVA, the negative charges are less frequent, thus lowering Pexiganan's adsorption; in turn, Tiger 17's adsorption was enhanced by means of electrostatic and hydrophobic interactions. Additionally, the high porosity characteristic of these electrospun fibers can also favorably contribute to the entrapment of Pexiganan, increasing its adsorption.⁵⁸ It is important to highlight the relationship between the porosity and peptide adsorption rates. Considering that the smallest diameters were obtained on the ratio 80/20 PVA/CNC, the smallest pores are expected to be obtained as well on this ratio. This data could indicate that Pexiganan can favorably be entrapped in fibers vertically, resulting in higher adsorption values (Figure 4 and Figure 6). In the C100/0 PVA/CNC and C80/20 PVA/CNC electrospun fibers, Tiger 17 presented the highest adsorption masses, which may indicate that the pore size may not influence its adsorption as much as the Pexiganan adsorption. The highest elastic shear moduli (~ 64 and 220 kPa, respectively) were also seen for Tiger 17 on C80/20 PVA/CNC mats, being that this stiffer adsorption may result from the pore size. In turn, Pexiganan showed a similar shear modulus for all electrospun mats, with 59, 58, and 80 kPa on C100/0, C90/10, and C80/20, respectively. Just like Tiger 17, the highest shear modulus was also seen on C80/20 mats, which may be related to its stiffer adsorption onto these mats as a response to pore size. Regarding the peptide's adsorption on the controls, the highest

ΔF drop was observed for Tiger 17, despite its lower concentration, while the ΔD variation remained close to zero. After the peptide's equilibrium, rinsing with dH_2O did not change ΔF , ΔD , or the elastic shear modulus, suggesting the irreversible attachment of both peptides onto the evaluated surfaces. It is worth highlighting that the peptide-induced ΔF and ΔD shifts were the highest in electrospun substrates compared to the controls (Figure 3). Considering that the peptides' adsorption profile onto all electrospun mats was indicative of the viscoelastic behavior (ΔD superior to 1×10^{-6} per 20 Hz), the Voigt model was used to translate the ΔF and ΔD shifts into adsorbed peptide masses (ng/cm^2) and elastic shear modulus (kPa) (Figures 4 and S3.1 and Table S3.1). The times (min) for reaching saturation were also measured (Figure 4 and Table S3.1).

It was seen that the surface properties effectively influence the adsorption of each structurally different peptide.³⁰ Hydrophobic interactions might occur between the acetate groups of PVA and hydrophobic amino acids in the peptides. The presence of one tryptophan and three cyclic prolines distributed consecutively between the lysine at the center of the Tiger 17 chain could have stimulated this type of interaction. In the case of Pexiganan, hydrophobic bonding may be generated via the majority of the hydrophobic amino acids that make up its chain, like glycine, alanine, valine, leucine, isoleucine, and phenylalanine (Table 1). The kinetics of peptide adsorption, in addition to being influenced by the diffusion coefficients and peptide structure, is also influenced by the peptide concentration.⁵⁹

Peptides at high concentrations with high diffusion coefficients tend to adsorb faster onto substrates due to a higher collision frequency.⁶⁰ This effect was noticeable on the control sensors since Pexiganan ($128 \mu\text{g}/\text{mL}$) required only ~ 41 min to reach equilibrium while Tiger 17 ($40 \mu\text{g}/\text{mL}$) took ~ 103 min. However, the opposite was observed on the electrospun fibers, with Tiger 17 requiring the least time to reach equilibrium. These results reinforce the idea that Pexiganan takes advantage of the nanofiber's larger surface area and porosity for establishing multisite bonding, thus requiring more time to completely bind to the surfaces. Consequently, Pexiganan achieved larger adsorption masses (Figure 4; details in Table S3.1). In contrast, the small, rigid, and cyclic structure of Tiger 17 hinders the adsorption between pores on electrospun nanofibers, yet it showed a high adsorption capacity in more uniform surfaces like spin-coated fibers.³⁰ Data prove the importance of a polymeric surface and

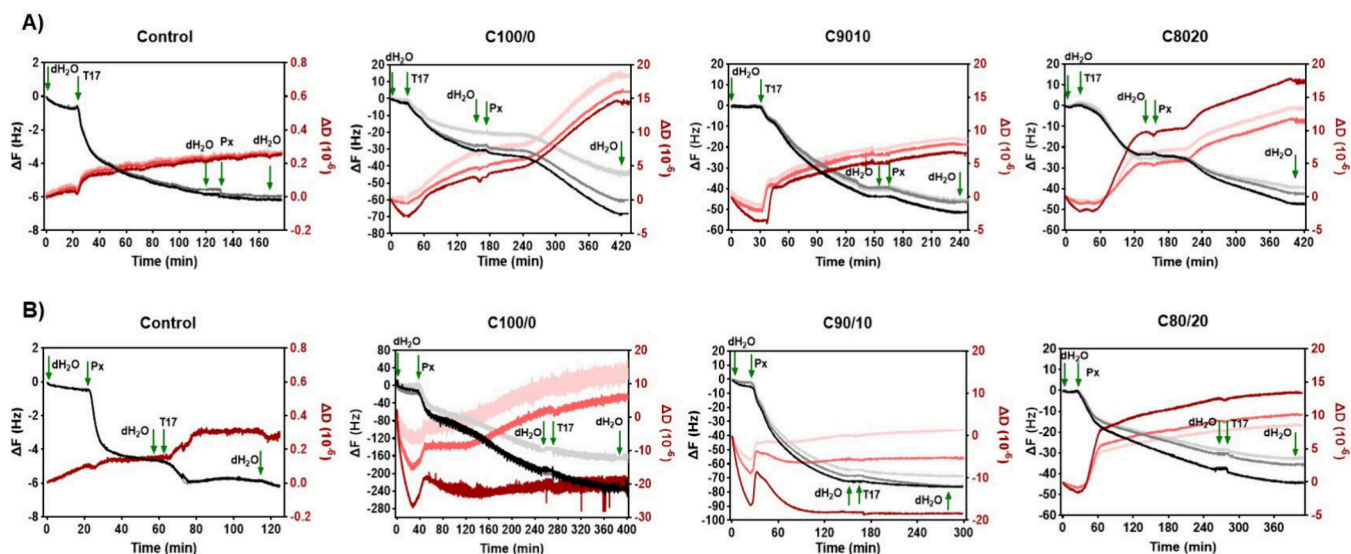


Figure 5. QCM-D frequency (ΔF) and dissipation (ΔD) plots of A) Tiger 17 (T17) + Pexiganan (Px) and B) Pexiganan (Px) + Tiger 17 (T17) sequential adsorption profiles onto QCM-D sensors without any coating (control group) and electrospun coated with C100/0, C90/0, and C80/20 PVA/CNC. 3rd, 5th, and 7th harmonics are represented; the higher the harmonics, the lighter the colors.

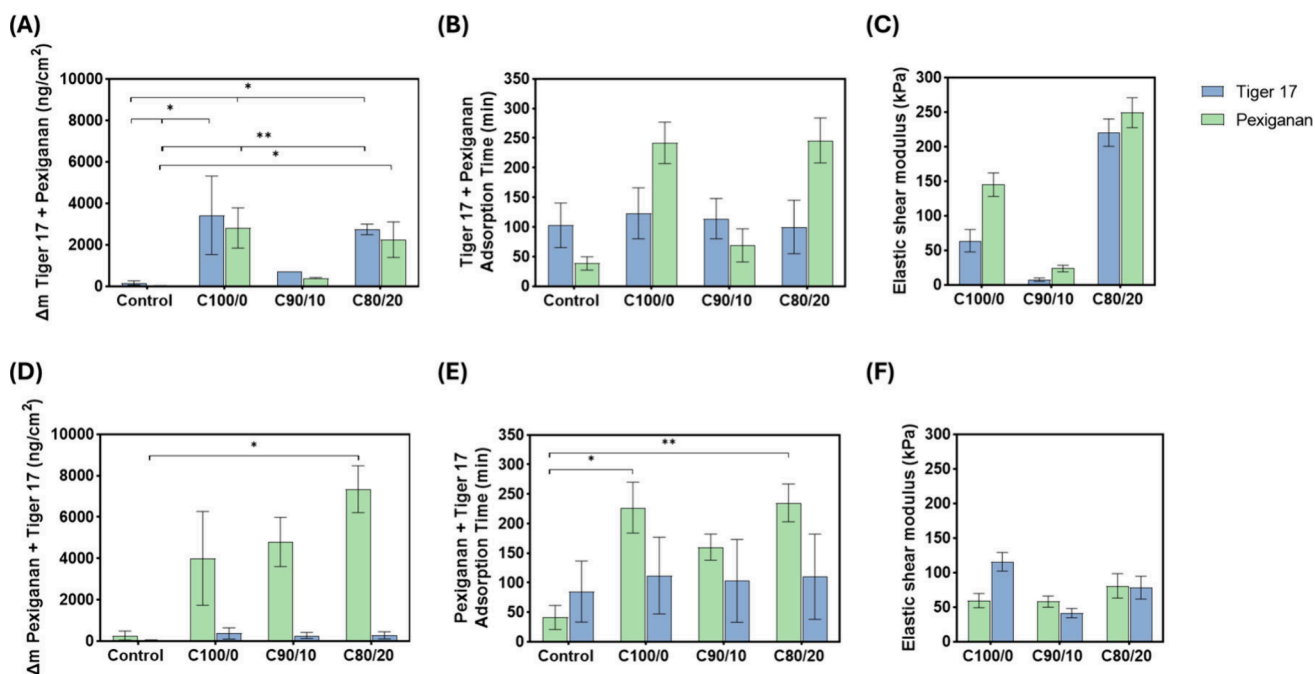


Figure 6. (A, D): Adsorbed mass (ng/cm^2) of Tiger 17 and Pexiganan from sequential adsorption measurements on control sensors and on C100/0, C90/10, and C80/20 PVA/CNC electrospun fibers. (B, E) Time needed for Tiger 17 and Pexiganan to reach surface saturation from sequential adsorptions. (C, F) Elastic shear modulus (kPa) for Tiger 17 and Pexiganan peptides in their adsorption equilibrium state. Statistical significance for (A), (B), (C), (D), (E), and (F) was determined via Kruskal-Wallis's test by applying the Dunn's test for multiple comparisons and was presented by * ($p \leq 0.05$), ** ($p \leq 0.01$).

high porosity levels to stimulate peptide binding. These facts were also proven by analysis and comparisons between the study developed by Lozeau et al. and the present study. Lozeau et al. demonstrated that cysteine-modified chrysophysin-1, a peptide similar to Pexiganan, achieved adsorption masses of between 0.8 and 2.2 μg on polyethylene glycol spacer-modified silicon QCM-D sensors, while on QCM-D sensors modified with polymeric electrospun mats the adsorption masses were higher, settling between 4.0 and 7.0 μg .⁶¹ Therefore, polymer

modifications and the presence of pores appear to be positive influences for increasing functionalization on these coatings.

Sequential-Peptide Adsorption Studies. The effect of the adsorption profile of one peptide on the adsorption of another was evaluated on control and PVA/CNC electrospun-coated sensors. Figure 5 shows the ΔF and ΔD shifts related to the peptides' sequential adsorption onto the sensors. These changes in ΔF and ΔD were converted into adsorbed peptide masses (ng/cm^2) and the time required to achieve equilibrium (Figure 6 and Table S3.2). As expected, the adsorption profiles

of both peptides when introduced as the first species were analogous to the results obtained from the individual studies (Figure 3).

The times to reach equilibrium as well as the elastic shear modulus were also similar. Therefore, this section will describe exclusively the adsorption of the second peptide onto an already preadsorbed peptide layer. Pexiganan introduced as the second peptide presented a ΔF larger than for Tiger 17 (introduced also as second peptide) on electrospun-modified surfaces, and, in general, the ΔD and elastic shear modulus were also greater. Compared to the outcomes as the first deposited peptide, when Pexiganan was the second peptide, there were reductions of $\sim 65\%$ on the control and ~ 30 , 92 , and 70% on the C100/0, C90/10, and C80/20 PVA/CNC-coated sensors, correspondingly. Similarly, when Tiger 17 was the second species in the sequence, the mass adsorption was reduced to $\sim 66\%$ on the control samples and to ~ 89 , 62 , and 90% on the C100/0, C90/10, and C80/20 PVA/CNC electrospun fibers, respectively. The Pexiganan introduced as the first peptide greatly hindered the subsequent adsorption of Tiger 17, particularly on polymeric mats (Figure 6c and Table S3.2). This is explained by the large chain length and linear structure of Pexiganan that promote multiple bondings on the substrate. When Tiger 17 was the second peptide, it did not displace the preadsorbed Pexiganan that has a greater affinity for CNC-containing fibers. However, the elastic shear modulus verified for Tiger 17, as the second peptide, remained relatively high (between 41 and 115 kPa), which confirmed its rigid structure as well as the formation of stable bonds with the surfaces.

In the sequence Tiger 17 + Pexiganan, the adsorption of each peptide was higher than in the sequence Pexiganan + Tiger 17, since Tiger 17 introduced as a second peptide achieved lower masses. Although the total peptide masses obtained in the sequence Pexiganan + Tiger 17 were the highest, 5068 and 7635 ng/cm² on C90/10 and C80/20 PVA/CNC, respectively, only $\sim 5\%$ of these masses were relative to Tiger 17. Since in CW treatments the presence of both peptides is essential to fight infections and promote the regeneration of tissues, an equal distribution of peptides was desirable. Data show that the sequence Tiger 17 + Pexiganan on C100% PVA and C80/20 PVA/CNC provides this desired outcome. C100/0 PVA/CNC fibers registered a total peptide mass of 6236 ng/cm² while C80/20 PVA/CNC reached 4985 ng/cm² with $\sim 55\%$ Tiger 17 and $\sim 45\%$ Pexiganan in both cases. This data highlights the ability of Pexiganan to be adsorbed within the spaces made available by Tiger 17. This was also denoted by the elastic shear modulus obtained for Pexiganan when introduced as a second peptide (Figure 6C). This means that despite some preadsorbed Tiger 17, Pexiganan effectively took advantage of the free spaces between the Tiger 17 molecules, contributing to the increase in its rigidity (shear modulus results, Figure 6C)), which may also indicate the formation of strong bonds with the surface.⁵⁴ It is worth mentioning that ΔF and ΔD shifts were larger in electrospun fiber-coated sensors than on controls, highlighting their attractiveness for peptide adsorption.

Thus, the results show that greater amounts of peptide can be adsorbed onto electrospun QCM-D sensors, and this is dependent on the order that peptides are introduced onto the surfaces as well as the composition of the polymeric substrate.

A schematic representation detailing the different scenarios for cyclic and linear peptides' adsorption on the electrospun fibers is presented in Figure 7.

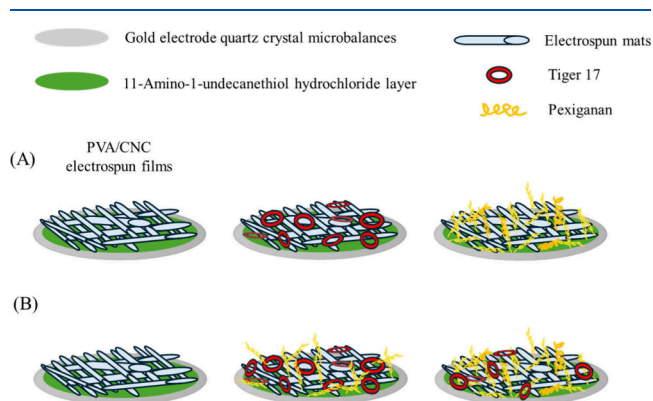


Figure 7. Schematic representation of the surface modifications performed on the QCM-D sensors on (A) single-peptide immobilization and (B) sequential immobilization on the engineered polymeric surfaces.

CONCLUSIONS

PVA-based electrospun fibers reinforced with 10 and 20% (w/v) CNC were successfully deposited on QCM-D sensors. The presence of PVA and CNC on the fibers was established by ATR-FTIR and contact angle measurements. Mats showed a hydrophilic profile, increasing the wettability of the surfaces as the CNC content was raised.

Electrospun fibers compared to the control (sensors without any modification) contributed the most to the adsorption of peptides. The linear and flexible Pexiganan achieved the higher adsorption masses on electrospun fibers as well as the highest dissipation values, particularly in the presence of CNC. These results revealed the importance of a large surface area and the presence of pores for peptide adsorption. Tiger 17 also appeared to be more inclined toward 100% PVA electrospun fibers since those contain the highest number of acetate groups available.

In sequential peptide adsorption studies, the sequence Pexiganan + Tiger 17 showed the highest total peptide adsorption on CNC-containing fibers. However, most of the peptide was composed of the first peptide. Thereby, a detailed analysis showed that the sequence Tiger 17 + Pexiganan on C100/0 PVA/CNC electrospun fibers was the most effective for the adsorption of the two peptides. It demonstrates that the polymeric anionic nature in physiological media instigated the adsorption of both peptides. However, it is worth highlighting that the CNC addition to the fibers not only raised the hydrophilic character of the substrates but also promoted the peptide adsorption, contributing to the establishment of strong bonds between the peptides and the surfaces, being a beneficial feature for an efficient CW treatment.

Data contributed to a deeper understanding of the influence of electrospun structures and their inherent hydrophobicity and hydrophilicity on the adsorption of peptides with different structures and compositions. More importantly, this study demonstrates a new potential strategy for CW care that combines electrospun nanofibers with antimicrobial and immunomodulatory peptides.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.langmuir.4c02825>.

Study of cross-linking efficiency; FTIR analyses; and peptide adsorption studies (PDF)

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Notes

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■ REFERENCES

- (1) Homem, N. C.; Miranda, C. S.; Teixeira, M. A.; Teixeira, M. O.; Domingues, J. M.; Seibert, D.; Antunes, J. C.; Amorim, M. T. P.; Felgueiras, H. P. Graphene Oxide-Based Platforms for Wound Dressings and Drug Delivery Systems: A 10 Year Overview. *J. Drug Deliv Sci. Technol.* **2022**, *78*, 103992.
- (2) Rianjanu, A.; Nugroho, D. B.; Kusumaatmaja, A.; Roto, R.; Triyana, K. A Study of Quartz Crystal Microbalance Modified with Polyvinyl Acetate Nanofiber to Differentiate Short-Chain Alcohol Isomers. *Sens Biosensing Res.* **2019**, *25*, 100294.
- (3) Teixeira, M. A.; Amorim, M. T. P.; Felgueiras, H. P. Cellulose Acetate in Wound Dressings Formulations: Potentialities and Electrospinning Capability. *XV Mediterranean Conference on Medical and Biological Engineering and Computing - MEDICON 2019*; Springer International Publishing, 2020; Vol. 76, pp 1515–1525.
- (4) Felgueiras, H. P.; Teixeira, M. A.; Tavares, T. D.; Amorim, M. T. P. New Method to Produce Poly(Vinyl Alcohol)/Cellulose Acetate Films with Improved Antibacterial Action. *Mater. Today Proc.* **2020**, *31*, S269–S272.
- (5) Luraghi, A.; Peri, F.; Moroni, L. Electrospinning for Drug Delivery Applications: A Review. *J. Controlled Release* **2021**, *334*, 463–484.
- (6) Teixeira, M. A.; Amorim, M. T. P.; Felgueiras, H. P. PVA/CA Based Electrospun Nanofibers: Influence of Processing Parameters in the Fiber Diameter. *IOP Conf Ser. Mater. Sci. Eng.* **2019**, *634* (1), 012040.
- (7) Vlad-Bubulac, T.; Hamciuc, C.; Rambu, C. M.; Aflori, M.; Butnaru, M.; Enache, A. A.; Serbezeanu, D. Fabrication of Poly(Vinyl Alcohol)/Chitosan Composite Films Strengthened with Titanium Dioxide and Polyphosphonate Additives for Packaging Applications. *Gels* **2022**, *8* (8), 474.
- (8) Huang, C.-Y.; Hu, K.-H.; Wei, Z.-H. Comparison of Cell Behavior on Pva/Pva-Gelatin Electrospun Nanofibers with Random and Aligned Configuration. *Sci. Rep* **2016**, *6* (1), 37960.
- (9) Unal, S.; Arslan, S.; Karademir Yilmaz, B.; Kazan, D.; Oktar, F. N.; Gunduz, O. Glioblastoma Cell Adhesion Properties through Bacterial Cellulose Nanocrystals in Polycaprolactone/Gelatin Electrospun Nanofibers. *Carbohydr. Polym.* **2020**, *233*, 115820.
- (10) Teixeira, M. A.; Paiva, M. C.; Amorim, M. T. P.; Felgueiras, H. P. Electrospun Nanocomposites Containing Cellulose and Its Derivatives Modified with Specialized Biomolecules for an Enhanced Wound Healing. *Nanomaterials* **2020**, *10* (3), 557.
- (11) Felgueiras, H. P.; Teixeira, M. A.; Amorim, M. T. P. Potentialities of LL37 for Wound Healing Applications: Study of Its Activity in Synergy with Biodegradable Composites Made of PVA and CA. *XV Mediterranean Conference on Medical and Biological Engineering and Computing - MEDICON 2019*; Springer International Publishing, 2020; Vol. 76, pp 1515–1525.
- (12) Felgueiras, H. P.; Amorim, M. T. P. Electrospun Polymeric Dressings Functionalized with Antimicrobial Peptides and Collagen Type i for Enhanced Wound Healing. *IOP Conf Ser. Mater. Sci. Eng.* **2017**, *254* (6), 062004.
- (13) Mahlapuu, M.; Björn, C.; Ekblom, J. Antimicrobial Peptides as Therapeutic Agents: Opportunities and Challenges. *Crit. Rev. Biotechnol* **2020**, *40* (7), 978–992.
- (14) Ladram, A.; Nicolas, P. Antimicrobial Peptides from Frog Skin %: Biodiversity and Therapeutic Promises. *Frontiers in Bioscience* **2016**, *21*, 1341–1371.
- (15) Lipsky, B. A.; Holroyd, K. J.; Zasloff, M. Topical versus Systemic Antimicrobial Therapy for Treating Mildly Infected Diabetic Foot Ulcers: A Randomized, Controlled, Double-Blinded, Multicenter Trial of Pexiganan Cream. *Clinical Infectious Diseases* **2008**, *47* (12), 1537–1545.
- (16) Tang, J.; Liu, H.; Gao, C.; Mu, L.; Yang, S.; Rong, M.; Zhang, Z.; Liu, J.; Ding, Q.; Lai, R. A Small Peptide with Potential Ability to Promote Wound Healing. *PLoS One* **2014**, *9* (3), e92082.
- (17) Khan, U.; Ryan, K.; Blau, W. J.; Coleman, J. N. The Effect of Solvent Choice on the Mechanical Properties of Carbon Nanotube-Polymer Composites. *Compos. Sci. Technol.* **2007**, *67* (15–16), 3158–3167.
- (18) Ajdnik, U.; Luxbacher, T.; Fras Zemljic, L. Proteins at Polysaccharide-Based Biointerfaces: A Comparative Study of QCM-D and Electrokinetic Measurements. *Colloids Surf. B Biointerfaces* **2023**, *221*, 113011.
- (19) Jackman, J. A.; Rahim Ferhan, A.; Cho, N.-J. Nanoplasmonic Sensors for Biointerfacial Science. *Chem. Soc. Rev.* **2017**, *46* (12), 3615–3660.
- (20) Felgueiras, H.; Migonney, V.; Sommerfeld, S.; Murthy, N. S.; Kohn, J. Competitive Adsorption of Albumin, Fibronectin and Collagen Type I on Different Biomaterial Surfaces: A QCM-D

Study. *XIII Mediterranean Conference on Medical and Biological Engineering and Computing* 2013; 2014; Vol. 41, pp 1597–1600.

(21) Jia, Y.; Yan, C.; Yu, H.; Chen, L.; Dong, F. One-Step Fabrication of Ammonia Sensor by Electrospinning PS-b-PMA Nanofibers on Quartz Crystal Microbalance. *Sens Actuators B Chem.* **2014**, *203*, 459–464.

(22) Aflaha, R.; Afiyanti, H.; Azizah, Z. N.; Khoirudin, H.; Rianjanu, A.; Kusumaatmaja, A.; Roto, R.; Triyana, K. Improving Ammonia Sensing Performance of Quartz Crystal Microbalance (QCM) Coated with Nanofibers and Polyaniline (PANi) Overlay. *Biosens Bioelectron X* **2023**, *13*, 100300.

(23) Nugroho, D. B.; Rianjanu, A.; Triyana, K.; Kusumaatmaja, A.; Roto, R. Quartz Crystal Microbalance-Coated Cellulose Acetate Nanofibers Overlaid with Chitosan for Detection of Acetic Anhydride Vapor. *Results Phys.* **2019**, *15*, 102680.

(24) Kurečić, M.; Mohan, T.; Virant, N.; Maver, U.; Stergar, J.; Gradišnik, L.; Kleinschek, K. S.; Hribernik, S. A Green Approach to Obtain Stable and Hydrophilic Cellulose-Based Electrospun Nanofibrous Substrates for Sustained Release of Therapeutic Molecules. *RSC Adv.* **2019**, *9* (37), 21288–21301.

(25) Nohut Maslakci, N.; Akalin, R. B.; Ulusoy, S.; Oksuz, L.; Uygun Oksuz, A. Electrospun Fibers of Chemically Modified Chitosan for in Situ Investigation of the Effect on Biofilm Formation with Quartz Crystal Microbalance Method. *Ind. Eng. Chem. Res.* **2015**, *54* (33), 8010–8018.

(26) Maslakci, N. N.; Ulusoy, S.; Oksuz, A. U. Investigation of the Effects of Plasma-Treated Chitosan Electrospun Fibers onto Biofilm Formation. *Sens Actuators B Chem.* **2017**, *246*, 887–895.

(27) Khoirudin, H.; Aflaha, R.; Arsetiyani, E. R.; Nugraheni, A. D.; Nurputra, D. K.; Triyana, K.; Kusumaatmaja, A. Influence of the SMN Antibody on Quartz Crystal Microbalance with Dissipation (QCM-D) Surface as an SMN Protein Biosensor. *MRS Commun.* **2024**, *14* (1), 34–40.

(28) Li, J.; Kaku, T.; Tokura, Y.; Matsukawa, K.; Homma, K.; Nishimoto, T.; Hiruta, Y.; Akimoto, A. M.; Nagase, K.; Kanazawa, H.; Shiratori, S. Adsorption-Desorption Control of Fibronectin in Real Time at the Liquid/Polymer Interface on a Quartz Crystal Microbalance by Thermoresponsivity. *Biomacromolecules* **2019**, *20* (4), 1748–1755.

(29) Şeker, Ş.; Elçin, A. E.; Elçin, Y. M. Real-Time Monitoring of Mesenchymal Stem Cell Responses to Biomaterial Surfaces and to a Model Drug by Using Quartz Crystal Microbalance. *Artif Cells Nanomed Biotechnol* **2016**, *44* (7), 1722–1732.

(30) Teixeira, M. A.; Murthy, N. S.; Ferreira, D. P.; Felgueiras, H. P. Comparison of the Adsorption of Linear and Cyclic Antimicrobial Peptides onto Cellulosic Compounds-Reinforced Poly(Vinyl Alcohol) Films Using QCM-D. *Colloids Surf. A Physicochem Eng. Asp* **2024**, *687*, 133490.

(31) Teixeira, M. A.; Antunes, J. C.; Seabra, C. L.; Fertzinhos, A.; Tohidi, S. D.; Reis, S.; Amorim, M. T. P.; Ferreira, D. P.; Felgueiras, H. P. Antibacterial and Hemostatic Capacities of Cellulose Nanocrystalline-Reinforced Poly(Vinyl Alcohol) Electrospun Mats Doped with Tiger 17 and Pexiganan Peptides for Prospective Wound Healing Applications. *Biomaterials Advances* **2022**, *137*, 212830.

(32) Teixeira, M. A.; Antunes, J. C.; Seabra, C. L.; Tohidi, S. D.; Reis, S.; Amorim, M. T. P.; Felgueiras, H. P. Tiger 17 and Pexiganan as Antimicrobial and Hemostatic Boosters of Cellulose Acetate-Containing Poly(Vinyl Alcohol) Electrospun Mats for Potential Wound Care Purposes. *Int. J. Biol. Macromol.* **2022**, *209*, 1526–1541.

(33) Tang, J.; Liu, H.; Gao, C.; Mu, L.; Yang, S.; Rong, M.; Zhang, Z.; Liu, J.; Ding, Q.; Lai, R. A Small Peptide with Potential Ability to Promote Wound Healing. *PLoS One* **2014**, *9* (3), e92082.

(34) Saftics, A.; Prósz, G. A.; Türk, B.; Peter, B.; Kurunczi, S.; Horvath, R. In Situ Viscoelastic Properties and Chain Conformations of Heavily Hydrated Carboxymethyl Dextran Layers: A Comparative Study Using OWLS and QCM-I Chips Coated with Waveguide Material. *Sci. Rep* **2018**, *8* (1), 11840.

(35) Solanki, P. R.; Arya, S. K.; Nishimura, Y.; Iwamoto, M.; Malhotra, B. D. Cholesterol Biosensor Based on Amino-Undecane-

thiol Self-Assembled Monolayer Using Surface Plasmon Resonance Technique. *Langmuir* **2007**, *23* (13), 7398–7403.

(36) Marcisz, K.; Karbarz, M.; Stojek, Z. Quartz Crystal Microbalance Electrode Modified with Thermoresponsive Crosslinked and Non-Crosslinked N-Isopropylacrylamide Polymers. Response to Changes in Temperature. *J. Solid State Electrochem.* **2016**, *20* (12), 3263–3270.

(37) Yilbas, B. S.; Al-Sharafi, A.; Ali, H. Surfaces for Self-Cleaning. *Self-Cleaning of Surfaces and Water Droplet Mobility*; Elsevier, 2019; pp 45–98.

(38) Peresin, M. S.; Habibi, Y.; Zoppe, J. O.; Pawlak, J. J.; Rojas, O. J. Nanofiber Composites of Polyvinyl Alcohol and Cellulose Nanocrystals: Manufacture and Characterization. *Biomacromolecules* **2010**, *11* (3), 674–681.

(39) Shaheen, Th. I.; Emam, H. E. Sono-Chemical Synthesis of Cellulose Nanocrystals from Wood Sawdust Using Acid Hydrolysis. *Int. J. Biol. Macromol.* **2018**, *107*, 1599–1606.

(40) Teixeira, M. A.; Amorim, M. T. P.; Felgueiras, H. P. Poly (Vinyl Alcohol) -Based Nanofibrous Electrospun Scaffolds for Tissue Engineering Applications. *Polymers* **2020**, *12*, 7.

(41) Semitela, A.; Girão, A. F.; Fernandes, C.; Ramalho, G.; Bdkin, I.; Completo, A.; Marques, P. A. Electrospinning of Bioactive Polycaprolactone-Gelatin Nanofibres with Increased Pore Size for Cartilage Tissue Engineering Applications. *J. Biomater Appl.* **2020**, *35* (4–5), 471–484.

(42) Wen, X.; Xiong, J.; Lei, S.; Wang, L.; Qin, X. Diameter Refinement of Electrospun Nanofibers: From Mechanism, Strategies to Applications. *Advanced Fiber Materials* **2022**, *4* (2), 145–161.

(43) Chen, W.; Gao, Z.; He, M.; Dou, Y.; Yin, G.; Ding, J. Vapor-Phase Glutaraldehyde Crosslinked Waste Protein-Based Nanofiber Nonwovens as an Environmentally Friendly Wound Dressing. *React. Funct. Polym.* **2022**, *172*, 105203.

(44) Sonker, A. K.; Rathore, K.; Nagarale, R. K.; Verma, V. Crosslinking of Polyvinyl Alcohol (PVA) and Effect of Crosslinker Shape (Aliphatic and Aromatic) Thereof. *J. Polym. Environ* **2018**, *26* (5), 1782–1794.

(45) Felgueiras, H. P.; Murthy, N. S.; Sommerfeld, S. D.; Brás, M. M.; Migonney, V.; Kohn, J. Competitive Adsorption of Plasma Proteins Using a Quartz Crystal Microbalance. *ACS Appl. Mater. Interfaces* **2016**, *8* (21), 13207–13217.

(46) Kuznetsov, G. V.; Islamova, A. G.; Orlova, E. G.; Ivashutenko, A. S.; Shanenkov, I. I.; Zykov, I. Y.; Feoktistov, D. V. Influence of Roughness on Polar and Dispersed Components of Surface Free Energy and Wettability Properties of Copper and Steel Surfaces. *Surf. Coat. Technol.* **2021**, *422*, 127518.

(47) Sadeghi-Aghbash, M.; Rahimnejad, M.; Adeli, H.; Feizi, F. Fabrication and Development of PVA/Alginate Nanofibrous Mats Containing Arnebia Euchroma Extract as a Burn Wound Dressing. *React. Funct. Polym.* **2022**, *181*, 105440.

(48) Safae-Ardakani, M. R.; Hatamian-Zarmi, A.; Sadat, S. M.; Mokhtari-Hosseini, Z. B.; Ebrahimi-Hosseinzadeh, B.; Rashidiani, J.; Kooshki, H. Electrospun Schizophyllan/Polyvinyl Alcohol Blend Nanofibrous Scaffold as Potential Wound Healing. *Int. J. Biol. Macromol.* **2019**, *127*, 27–38.

(49) Barberi, J.; Spriano, S. Titanium and Protein Adsorption: An Overview of Mechanisms and Effects of Surface Features. *Materials* **2021**, *14* (7), 1590.

(50) López-córdoba, A.; Estevez-areco, S.; Goyanes, S. Potato Starch-Based Biocomposites with Enhanced Thermal, Mechanical and Barrier Properties Comprising Water-Resistant Electrospun Poly (Vinyl Alcohol) Fibers and Yerba Mate Extract. *Carbohydr. Polym.* **2019**, *215* (November 2018), 377–387.

(51) Popescu, M.; Dogaru, B.; Goanta, M.; Timpu, D. Structural and Morphological Evaluation of CNC Reinforced PVA/Starch Biodegradable Fibers. *Int. J. Biol. Macromol.* **2018**, *116*, 385–393.

(52) Park, Y.; You, M.; Shin, J.; Ha, S.; Kim, D.; Heo, M. H.; Nah, J.; Kim, Y. A.; Seol, J. H. Thermal Conductivity Enhancement in Electrospun Poly(Vinyl Alcohol) and Poly(Vinyl Alcohol)/Cellulose Nanocrystal Composite Nanofibers. *Sci. Rep* **2019**, *9* (1), 3026.

- (53) Destaye, A. G.; Lin, C.; Lee, C. Glutaraldehyde Vapor Cross-Linked Nanofibrous PVA Mat with in Situ Formed Silver Nanoparticles. *ACS Appl. Mater. Interfaces* **2013**, *5*, 4745.
- (54) Malmström, J.; Agheli, H.; Kingshott, P.; Sutherland, D. S. Viscoelastic Modeling of Highly Hydrated Laminin Layers at Homogeneous and Nanostructured Surfaces: Quantification of Protein Layer Properties Using QCM-D and SPR. *Langmuir* **2007**, *23* (19), 9760–9768.
- (55) Hajiraissi, R.; Hanke, M.; Yang, Y.; Duderija, B.; Gonzalez Orive, A.; Grundmeier, G.; Keller, A. Adsorption and Fibrillization of Islet Amyloid Polypeptide at Self-Assembled Monolayers Studied by QCM-D, AFM, and PM-IRRAS. *Langmuir* **2018**, *34* (11), 3517–3524.
- (56) Ehmman, H. M. A.; Mohan, T.; Koshanskaya, M.; Scheicher, S.; Breitwieser, D.; Ribitsch, V.; Stana-Kleinschek, K.; Spirk, S. Design of Anticoagulant Surfaces Based on Cellulose Nanocrystals. *Chem. Commun.* **2014**, *50* (86), 13070–13072.
- (57) Liu, S. X.; Kim, J.-T. Application of Kevin–Voigt Model in Quantifying Whey Protein Adsorption on Polyethersulfone Using QCM-D. *JALA: Journal of the Association for Laboratory Automation* **2009**, *14* (4), 213–220.
- (58) Cohen, H.; Wani, N. A.; Ben Hur, D.; Migliolo, L.; Cardoso, M. H.; Porat, Z.; Shimoni, E.; Franco, O. L.; Shai, Y. Interaction of Pexiganan (MSI-78)-Derived Analogues Reduces Inflammation and TLR4-Mediated Cytokine Secretion: A Comparative Study. *ACS Omega* **2023**, *8* (20), 17856–17868.
- (59) Liu, J.; Lee, M. L. Permanent Surface Modification of Polymeric Capillary Electrophoresis Microchips for Protein and Peptide Analysis. *Electrophoresis* **2006**, *27* (18), 3533–3546.
- (60) Liu, J.; Lee, M. L. Permanent Surface Modification of Polymeric Capillary Electrophoresis Microchips for Protein and Peptide Analysis. *Electrophoresis* **2006**, *27* (18), 3533–3546.
- (61) Lozeau, L. D.; Alexander, T. E.; Camesano, T. A. Proposed Mechanisms of Tethered Antimicrobial Peptide Chrysopsin-1 as a Function of Tether Length Using QCM-D. *J. Phys. Chem. B* **2015**, *119* (41), 13142–13151.