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Characterization of peptide coatings adhered to synthetic fibers: A versatile model for peptide nucleic acids

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ABSTRACT

Peptide nucleic acids (PNAs) are an emerging family of biomaterials designed to specifically target and treat diseased cells, most commonly in the antimicrobial-resistant bacteria MRSA. While PNAs offer great promise for the elimination of these bacteria, they are significantly more expensive than traditional peptides and often do not provide functionality for analysis. In this work, a model peptide (KFFCCQ) was developed to evaluate peptide coatings adhered to fibrous surfaces with multiple functional handles, i.e. the presence of a sulfur atom in cysteine and an aromatic ring in phenylalanine, to predict the durability of PNA coatings on 50/50 nylon/cotton blends (NyCo), which are commonly used in clothing such as combat and medical attire. Following elemental analysis through XPS and EDX-SEM, rinses were performed on the fabrics and the subsequent release of peptide was evaluated with UV–vis. As expected, elevated temperature and increased time resulted in higher KFFCCQ release levels from the NyCo fibers. Finally, EDX-SEM examined the presence of KFFCCQ following rinse cycles, revealing that a higher level of KFFCCQ released from nylon fibers compared to cotton fibers. This evaluation proves the utility of KFFCCQ as a preliminary model to evaluate adhesion and release of peptides from nylon and cotton fibrous surfaces.

Textiles are a vital component to modern-day life and efficient modification of both synthetic and natural fibers is a crucial science and engineering concern. Most notably, dyes, antibacterials, and stain-resistant coatings must both adhere to woven fibers and survive external aqueous environments and mechanical agitation [1–6]. These coatings range in function from color fade resistance to therapeutic use [6, 7]. Recently, silver nanoparticles and tailored polymers as antibacterial coatings on fabrics justify the importance of developing and understanding biomaterial coatings and release profiles [3, 5, 8]. The characterization of fiber coatings and their resistance to multiple aqueous washing cycles is crucial to evaluate any new potential textile.

Although traditional antibacterial coatings offer precedence, they are often deemed too broad a class of antimicrobials, leading to the subsequent loss of naturally occurring bacteria on skin [8]. Peptide nucleic acids (PNAs) are an emerging biologic moiety that possess the potential to selectively eradicate numerous diseases [9–13]. DNA base pairs covalently attached to a peptide backbone reminiscent of proteins compose PNAs, harnessing the ability to combine the therapeutic function of DNA/RNA and protein [9]. Their action varies on the specific application, but their main attraction is their specificity to target organisms. For example, Sato et al. described the use of PNAs as siRNA carriers to deliver these sensitive genes directly to the nucleus of cells [14]. More commonly, drug-resistant bacteria, such as Methicillin-resistant Staphylococcus aureus (MRSA), requires PNAs for treatment [12]. These PNAs eliminate specific targets within MRSA, such as FtsZ, allowing other bacteria to survive [12].

Due to the high cost and often difficult characterization of PNA samples, developing simpler and cheaper model peptides to study the adhesion to fibers is important. PNAs typically contain two separate functional sequences in the backbone. First is the cell-penetrating peptide (CPP) sequence, typically incorporating high amounts of cationic charge through lysine or arginine incorporation. Second is the active inhibiting sequence, specific to targeting pathways or signals from a particular target, such as MRSA. Therefore, our unique design of a model peptide contains both a lysine residue to mimic charge

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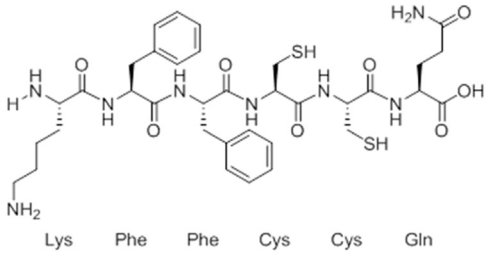

Fig. 1. Model peptide consisting of Lysine-Phenylalanine-Phenylalanine-Cysteine-Cysteine-Glutamine (KFFCCQ) sequence.

density, as well as cysteine and phenylalanine for easy analytical analysis. This yielded a peptide with similar charge density to PNAs, while maintaining the peptide backbone suitable for coating and adhesive analysis (KFFCCQ, EnoGene Biotech Co Ltd) [15, 16]. UV–vis spectroscopy detected phenylalanine residues while elemental analysis, such as X-ray photoelectron spectroscopy (XPS, PHI Quantera SXM) or scanning electron microscopy (SEM, FEI Quanta 600 FEG) equipped with electron dispersive X-ray spectroscopy (EDX, Bruker with Silicon Drifted Detector), identified sulfur atoms present in the cysteine residues [17] (Fig. 1).

XPS analysis of neat fabrics confirmed the lack of sulfur atoms present in commercially available 100% cotton, 100% nylon, or a 50/50 nylon/cotton (NyCo) blend, as shown in Table 1. All fibers showed a silicon signal, likely arising from a protective coating from a pre-treatment. NyCo samples reveal a magnesium peak, likely arising from dye or coating used on the fabric as demonstrated by a lack of magnesium upon bleach treatment [18]. Elemental analysis proves crucial to confirm KFFCCQ presence after coating procedures. Following characterization of neat fibers and validation of the lack of interference in elemental signal from the fibers, the NyCo sample was identified for detailed study.

Pad-dry and pad-dry-cure techniques proved viable methods to coat KFFCCQ to NyCo fibers [7, 19, 20]. To compare these techniques, NyCo was soaked in aqueous 0.1 wt % or 0.5 wt % KFFCCQ solution for 30 min, with gentle agitation every 10 min. Pad-dry proceeded to dry the samples at 95 °C for 1 h, while pad-dry-cure placed the samples at 95 °C for 90 s followed by 150 °C for 60 s. Both pad-dry and pad-dry-cure methods sufficiently adhered the KFFCCQ to the 50/50 NyCo blend, with no discernible difference noticed between techniques. Table 2 demonstrates changes in global elemental compositions (through XPS analysis) of fibers after adhering the peptide coating with varying weight percent KFFCCQ. As expected, 0.5 wt % KFFCCQ elicited higher sulfur content than 0.1 wt %, indicating a higher incorporation. The lack of variation between pad-dry and pad-dry-cure techniques for KFFCCQ incorporation suggests utility in the reduced-time pad-dry-cure method notwithstanding potential changes in function [7].

A broad S2p binding energy peak at 164 eV confirmed the retention of cysteine residues adhered to fiber surfaces following 0.5 wt % pad-dry and pad-dry-cure procedures, as shown in Fig. 2 [21].

SEM provided further confirmation of the utility of both pad-dry and pad-dry-cure techniques as compared to neat NyCo fibers. As seen in Fig. 3a, cotton presented as rough, twisting fibers, while nylon presented smooth, cylindrical fibers. As seen in Fig. 3b-d, the surface morphology of both fiber types after coating suggested successful adhesion of KFFCCQ throughout the NyCo sample. Regions of pad-dry samples exhibited bridging between fibers, while pad-dry-cure samples retain well-defined morphology. These images indicate slight differences in both KFFCCQ content as well as coating morphology, suggestive of nuanced differences between the techniques not seen with XPS.

Table 2
XPS analysis of NyCo samples treated with 0.1 and 0.5 wt% model peptide solutions, undergoing both pad dry and pad dry cure methods.

Sample	C1s	N1s	O1s	Si2p	S2p	Cl2p
0.1 wt % Pad Dry	76.68	2.91	18.26	2.16	0	0
0.1 wt % Pad Dry Cure	80.24	4.20	14.06	0.86	0.41	0.22
0.5 wt % Pad Dry	74.01	5.40	17.43	2.14	0.73	0.30
0.5 wt % Pad Dry Cure	79.86	4.36	13.35	1.52	0.71	0.20

In conjunction with SEM imaging, EDX elemental analysis confirmed the presence of sulfur atoms on fiber surfaces after coating, as shown in Fig. 6. Fig. 4 depicted neat NyCo fibers and revealed a lack of sulfur present in the sample, in concordance with XPS performed on neat fabric. EDX provided insight into elemental composition across the entire depth of the fabric sample, as a thin coating present only on the surface would not produce a detectable signal. XPS and EDX together provided evidence that KFFCCQ is adhering to and coating both nylon and cotton fibers throughout the bulk of the sample.

The phenylalanine residues in KFFCCQ provide a metric to quantitatively determine release of KFFCCQ from NyCo fibers after aqueous rinsing. Absorbance of phenylalanine at 237 nm in water yielded a concentration-dependent absorption profile, which accurately probed samples of low concentration. The absorbance profile up to 2 mg/mL follows the linear fit

$$Absorbance = 0.2818c + 0.0317$$

where c indicates the concentration of KFFCCQ in mg/mL. KFFCCQ follows a linear fit at dilute concentrations, affording a method sensitive to low concentrations of peptide.

KFFCCQ-loaded NyCo was prepared by soaking in aqueous 0.5 wt % solution of KFFCCQ for 30 min with agitation every 10 min, followed by drying at 55 °C for 1 h. Dried, coated fabrics were rapidly stirred at 44 °C, 25 °C, or 16 °C in water for 0, 16, or 21 min. UV–vis absorption (Fig. 5**)** provided quantitative analysis of released KFFCCQ following lyophilization of collected water samples (n=3). KFFCCQ-loaded fabrics subjected to this rinse cycle exhibited a loss of peptide, with a rinse cycle performed at 44 °C for 16 min providing the most significant release of KFFCCQ from NyCo fibers, indicating a rapid release from fibers. Furthermore, NyCo subjected to a rinse cycle at 16 °C and 25 °C show marginal release from NyCo fibers, indicating warmer water disrupts KFFCCQ binding to a higher degree than colder water, as adhesion is most likely due to hydrogen bonding. Future studies

Table 1
XPS analysis of Cotton (neat & autoclaved), Nylon (neat & autoclaved), and 50/50 Nylon/Cotton (NyCo) (neat, autoclaved, and bleached) revealing the presence of coatings and dyes. XPS confirms the utility in the model peptide through the lack of elemental interference from fibers.

Sample	C1s	N1s	O1s	F1s	Na1s	Mg2s	Si2p	P2p	S2p	Cl2p	Ca2p
Cotton	74.95	0.33	24.65	0	0.14	0	0.51	0	0.19	0	0.23
Cotton (Autoclaved)	71.63	1.30	22.73	2.41	0.13	0	0.97	0.26	0	0	0.57
Nylon	74.11	9.44	14.73	0	0	0	1.38	0	0	0	0.34
Nylon (Autoclaved)	70.4	8.63	18.07	0.42	0	0	2.23	0	0	0	0.24
NyCo	83.43	1.72	13.31	0	0	0.44	0.54	0	0	0.15	0.40
NyCo (Autoclaved)	78.03	3.18	15.84	0	0	0.34	2.31	0	0	0	0.30
NyCo (Bleached)	74.96	5.87	11.71	0	0.88	0	0.41	0	0.14	6.04	0