Doxyclycline and Ciprofloxacin Treatment of Silk to Produce Novel Infection Resistance Biomaterials

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Abstract

Two antibiotics, doxycycline (doxy) and ciprofloxacin (cipro) were applied under a variety of conditions to silk, and to silk that had previously been hydrolyzed. FTIR-ATR analyses indicated that the drastic increase in sorption of antibiotics by hydrolyzed silk was attributable to both chemical and conformational changes that occurred on hydrolysis. A high sorption of doxy by hydrolyzed silk did not necessarily give a more infection-resistant material as determined by a zone of inhibition test. Conversely, the same hydrolysis considerably increased both sorption of cipro and zone of inhibition of cipro-treated silk dyed at 65°C and 85°C.

Introduction

One of the biggest problems in implantation of materials in vivo is bacterial infection caused by the material implanted.[1] Combating such infections relies on the use of antibiotics, many of which have molecular masses and structural features that render them superficially similar to textile dyes.[2] The similarity is close enough to be of practical use in developing materials with improved infection-resistant properties by using dyeing technology.

Thus polyester and polyurethane materials have been "dyed" with fluoroquinolone antibiotics.[2-6] The interactions are of the right order such that the antibiotics released slowly and continuously from the substrate, providing antibiotic activity that is maintained over periods of time far in excess of those obtained simply by "dipping" the material in a solution of antibiotic.[3] Implantation devices and sutures are dipped or soaked in a solution of penicillin and heparin at the operating table immediately prior to insertion in the patient to prevent any infection or other complications such as thrombosis.[2,6] Such attempts have not been completely effective, because of the brief residence of antibiotics at the implantation site.[3] Therefore, sustained release of antibiotics becomes important to keep the implantation site bacteriostatic for a longer period of time.

Unlike other common biomedical textiles such as polyester, silk contains various polar functional groups that might enhance the sorption of antibiotics.[1] The same functional groups might change the release characteristics at the same time: for better (greater interaction leading to a slower release and longer efficacy) or worse (the interactions might be great enough to prevent sufficient release for antibiotic activity to be sustained). The efficacy might also be modified by the conditions of application that could lead to antibiotic diffused throughout the fiber, or confined more to the fiber surface. These considerations prompted this study of the interaction of antibiotics with silk. In this work we "dyed" silk fabrics with two antibiotics, doxycycline (doxy) and ciprofloxacin (cipro).

Experimental

The dyeing of antibiotics on silk was carried out in an Ahiba Polymat (Datacolor International) dyeing machine. 2% on the weight of fabric (owf) antibiotic was applied at a liquor-to-fabric ratio of 20:1. Experimental parameters were dyeing temperature, time, and dyebath pH. After the process was run, the fabric was removed, and the amount of antibiotic taken up by the silk was determined.

To investigate effect of hydrolysis on sorption of antibiotics, the silk was treated in 1% NaOH for 20, 40, and 60 min at 40°C and 20:1 liquor ratio in the Ahiba dyeing machine.

To determine sorption of antibiotics by silk, the concentration of residual antibiotics in the dyebath after dyeing was measured using a Cary 50 UV/VIS spectrophotometer (Varian Instruments, USA). The data were analyzed in terms of this residual concentration. The CIE lightness (L*) of doxy-dyed silk fabric was evaluated with a Macbeth ColorEye system. The infrared spectra of silk were obtained with a Sense FTIR spectroscope

(SensIR Technologies) with an attached diamond ATR in the spectral region of 4000-700 cm^{-1} with 54 scans at 4 cm^{-1} resolution.

A zone of inhibition test determined the antimicrobial activity of the dyed materials against *S. epidermidis* as described in the previous study.[2]

Results and Discussion

Effect of temperature and time on sorption of doxy

Sorption of doxy on silk was essentially consistent at different dyeing temperatures. The concentration of residual doxy was somewhat less at 100°C, but this was due to the decomposition of doxy at 100°C as shown by the loss of concentration in the blank bath (no silk). The presence of silk tended to alleviate decomposition of doxy at high temperature.

Since both silk fibroin and doxy are amphoteric in nature with isoelectric points of zwitterions at around 5,[4] it was initially thought that sorption of doxy on silk would be largely dependent upon bath pH. However, sorption of doxy varied little with pH, and the only effect observed was a higher sorption and exhaustion of doxy at pH 2. This result suggested that other interactions such as hydrogen bonding play a greater role in sorption of doxy.

Effect of temperature on cirpo sorption

The thermal stability of cipro is better than that of doxy, indicated by the consistent absorbance of a blank bath at λ_{max} at 100°C and no color change. It also has lower aqueous solubility than doxy, being only soluble in acidic pHs at room temperature.[6] Among the three pHs examined in the current study, complete dissolution only occurred at pH 3 where it has the maximum stability.[6] It is therefore expected that pH would show more effect on the sorption of cipro than of doxy.

The sorption of cipro at pH 3 did not vary significantly with dyeing temperature. Even at 100°C, the exhaustion of cipro was less than 20% at pH 3. However, at pH 5.5 and 10 concentrations of residual cipro were considerably lower at 45°C than those at higher temperatures. Extremely low residual concentrations of cipro in the dyebath at 45°C, and pHs 5.5 and 10 were thought to be mainly due to insolublization of cipro during dyeing. Cipro precipitated by the pH adjustment was physically trapped within the fabric structure resulting in low measured concentration of residual cipro in the bath.

Antibiotic dyeing of hydrolyzed silk

Hydrolyzed silk showed drastically increased sorption of doxy and cipro at all three temperatures examined. Percent exhaustions of antibiotics on silk hydrolyzed for 60 min were between 86.3% and 90.1% for doxy and 78.2% and 82.4% for cipro compared to corresponding blank solution. These results clearly demonstrated that highly improved sorption and % exhaustion of the antibiotics such as doxy and cipro could be obtained by hydrolysis of silk.

FTIR-ATR analyses suggested that substantial increase in sorption of antibiotics in hydrolyzed silk was due to a combination of several factors such as increase of polar functional groups and amorphous regions.

Infection resistance properties of the treated silk

Somewhat surprisingly the silk fabrics dyed at lower temperatures (45°C and 65°C) had a more prolonged release of doxy. It is expected that even with the same level of antibiotic on the fiber, the location of antibiotic within the fiber structure would vary with dyeing temperature. Thus at 100°C doxy was diffused more deeply within the fiber, and once the leaching of surface-bound doxy was complete within four hours, little activity remained.

Contrarily, the zone of inhibition of the cipro-treated silk decreased quickly with washing time and no activity remained beyond four hours for any pH of application. We therefore suspected that interactions of doxy and cipro with silk were quite different, resulting in different location within the fiber, or a lower rate and/or extent of diffusion from the material under the conditions of the zone test.

References

1. G. Altman, F. Diaz, C. Jakuba, T. Calbro, R. Horan, J. Chen, H. Lu, J. Richmond, and D. Kaplan, *Biomaterials* **24(3)**, 401-416 (2003).
2. C. Yuan, M. Bide, M. Phaneuf, W. Quist, and F. LoGerfo, *AATCC Reviews* **1(8)**, 35-39 (2001).
3. C. L. Fox, S. Modak, and K. Reemtsma, *US patent* 4,563,485, Jan 7, 1986.
4. R. Bocker, *J. of Chromatography* **187**, 439-441 (1980).
5. A. Haverich, S. Hirt, M. Karck, F. Sclari, and H. Wahlig, *J of Vascular Surgery* **15(1)**, 187-93 (1992).
6. K. Torniainen, S. Tammilehto, and V. Ulvi, *International J of Pharmaceutics* **132**, 53-61 (1996).

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