A New Method of Detecting Damaged Collagen in Parchment Using Collagen Hybridizing Peptide

Ye Rin Kim[1], Michael S. Yu[2], Patricia McGuiggan[1]

[1] Department of Material Science and Engineering, Johns Hopkins University, [2] Department of Biomedical Engineering, University of Utah

Johns Hopkins University | Whiting School of Engineering | Baltimore, MD

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Introduction

Conservation is the preservation of artistic, historic, and cultural materials. Protecting objects now is the only way to ensure that future generations will be able to appreciate and learn from intact historical media. Exploration of new technology can give rise to new techniques used in conservation. This project utilizes collagen hybridizing peptide (CHP), which is typically used to identify and visualize denatured collagen in tissue samples. CHP will be used in order to develop a new method of characterizing damage in parchment.

Objectives

The purpose of this project is to design a set of experiments that will establish a protocol of using CHP to analyze collagen denaturation on parchment. In order to do so:

- Verify that CHP adheres to parchment.
- Compare CHP with other standard techniques used in conservation to characterize damage in parchment.
- Examine if CHP is a viable treatment method for parchment.

Materials and Methods

The parchments primarily used in this study – sheep (A) and calfskin (B) – were sourced from Talas. Whatman paper was used as a non-collagenous control. Goatskin hide being processed into parchment was sourced from Pergamena. F-CHP was sourced from 3Helix.

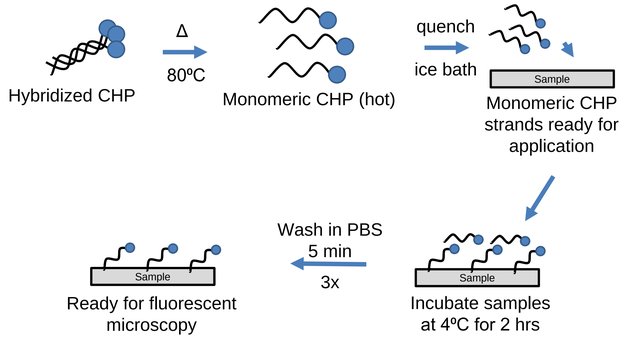


Figure 1—Application of CHP to sample following 3Helix's F-CHP protocol for staining histology slides.

20uL of 20uM F-CHP (3Helix), diluted with 1x PBS buffer was heated to 80°C, quenched to room temperature, then added to the samples. After sitting for 2 hours at 4°C, the samples were washed with PBS. Fluorescence microscopy was taken using a handheld MiScope Megapixel2 and Zeiss Axiovert 200 LM. Sample surface images were taken using an intermittent/tapping mode AFM cantilever (Bruker Nano).

Samples were artificially aged in an oven at 110°C for 96 hours, one set in dry conditions and in the other humid. FTIR of the samples were taken using the Bruker Alpha FT-IR Spectrometer at 0, 24, and 96 hours in order to observe the chemical changes taking place during artificial aging.

Results

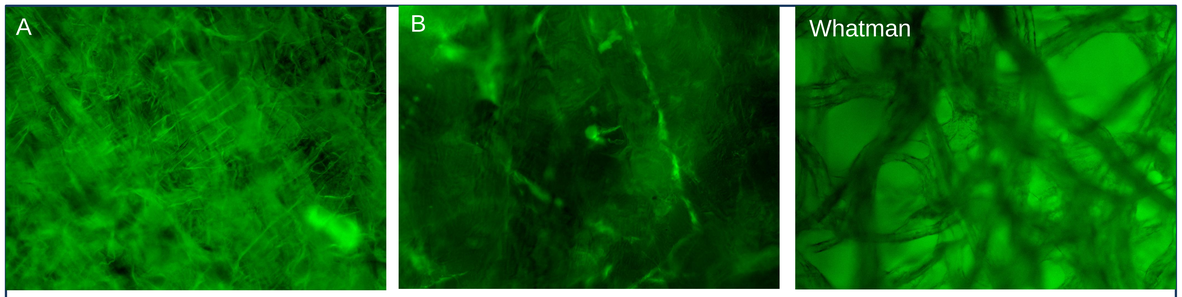


Figure 2—20x Air Microscopy images of A (left), B (middle), and Whatman (right).

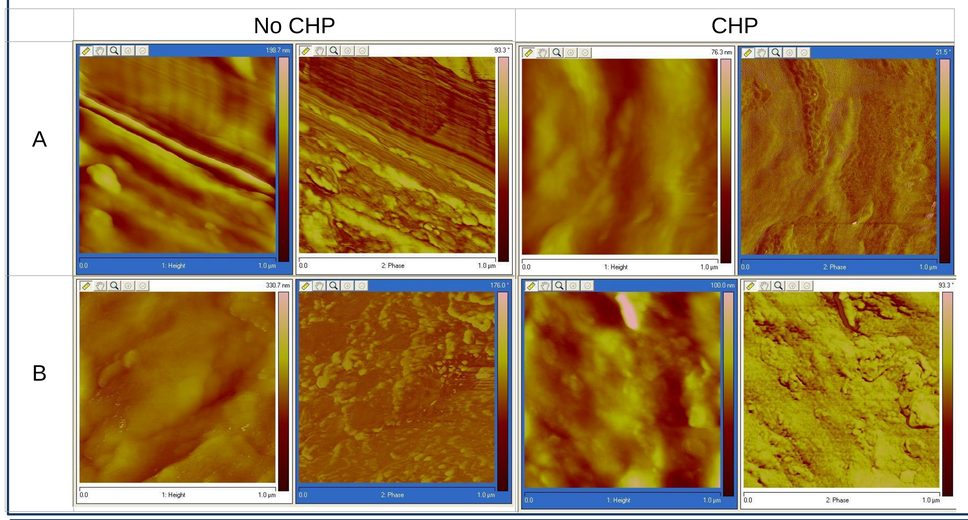
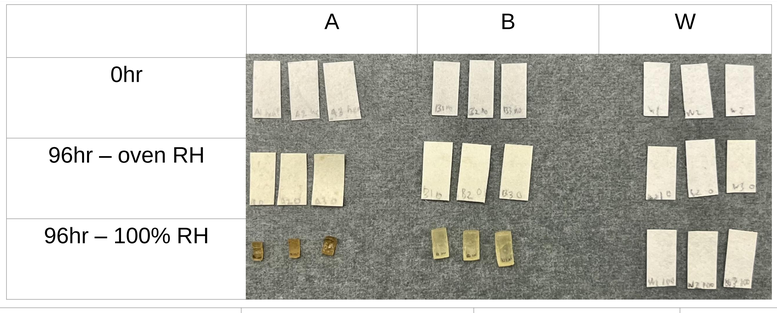


Figure 3— AFM surface images taken of Parchment A and B before and after CHP is applied. Sample area: 1.0µm x 1.0µm



	Original	After Aging	%Shrinkage
A (sheep) | oven RH	0.5 x 1cm	-	No change
| 100% RH	0.5 x 1cm	0.4 x 0.17 cm	13.6%
B (calf) | oven RH	0.5 x 1cm	-	No change
| 100% RH	0.5 x 1cm	0.3 x 0.6 cm	36%
Whatman | oven RH	0.5 x 1cm	-	No change
| 100% RH	0.5 x 1cm	-	No change

Figure 4—Measuring %shrinkage of samples after artificial aging in oven.

Conclusions

This study was able to establish that CHP will adhere to the denatured collagen in parchment. We had concerns about interaction occurring between the dye and parchment as well as over-signaling due to damage caused by the parchment manufacturing process, but CHP must likely will be viable as a method to detect denatured collagen.

The AFM microscopy revealed that the surface topology of parchment A and B were altered during the process of applying CHP. Parchment A was layered while parchment B had a more flat, crystalline structure pre-application, while both parchment surface topologies were more smoothed out after CHP was applied. We attribute the change due to the time the samples spent soaked in PBS, as well as possibly salt in the PBS being left as residue on parchment. AFM was attempted as possibly an alternate way of looking at the presence of CHP on the samples, but we aren't sure what CHP would exactly look like in the topography or if it is even large enough to be imaged. We were interested in CHP as a non-destructive method of characterizing parchment damage but the change in parchment structure is slightly concerning, as conservation aims for minimal change occurring to the object.

Artificially aging the samples under these conditions revealed that the most drastic changes occurred in the high humidity environment. Water and to some degree, heat, plays a large role in the denaturation of collagen. Samples A and B in the 100% RH environment underwent a significant color change, shrank, hardened, and became thicker. In contrast, Whatman paper underwent very little change. In the oven RH, both parchments A and B browned, indicating some chemical change occurred but no change in area occurred. We hypothesize if CHP were to be applied to both dry and humid aged samples, both sets of samples would have increased fluorescent signal, with the 100%RH samples fluorescing the most.

Future Works

- Damage caused by parchment during parchment-making process
- CHP as a parchment treatment
- Applying CHP to other collagenous material

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