STUDY ON THE DIFFERENCE OF COLLAGEN FIBRE STRUCTURE CAUSED BY EPOXY RESIN EMBEDDING

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Abstract. The researches on collagen that possesses unique fibre structure are reported frequently. In this paper, the cross images of leather fibre of dried wet blue cowhide leather embedded with and without epoxy resin(E51) were investigated with the micro computed topography(MCT). The images obtained by MCT of leather fibre are original status without any damage, while the embedded leather can emerge distortion because the fibre was fixed during the solidifying and immersing of the resin. In this research, 1178 images of leather fibre were investigated on wet blue leather(original fibre) and the same piece of leather embedded by epoxy resin(embedded fibre). The total pixels number of the sections from the original fibre and the embedded fibre was examined for each image. The results showed that the fiber contraction rate of the embedded skin sample calculated by the MATLAB method is 7.93%, and the overall "panel" contraction rate of the embedded skin sample is 10.88%.The embedded skin sample has significant shrinkage. Otherwise, different denoising methods will produce different effects. In other words, there may be two problems of insufficient or excessive denoising. This will result in a deviation and it needs further analysis.

1 Introduction

Collagen is widely used in medical and cosmetic fields1, so it is of great significance to study its internal structure. Collagen is the main component of animal support structure. As a natural polymer material, collagen has unique fiber structure, which is incomparable with other materials. Type I collagen accounts for 90% of total collagen and 20% of total protein2. Many studies have shown that collagen molecules are extremely long, in relation to their cross-section, which are triple helix structures with a length of about 280nm, a diameter of 1.4 ~ 1.5nm, and a molecular weight of about 300,0003. By electron microscopy, the collagen fibrils were observed to have light and dark periodic transverse striations of about 60-68nm (D-spacing), and the lateral accumulation of microfibril was fibril with a diameter of 30-500nm which changes with the animal species, tissue origin and age4. However, the pattern of fibril forming fiber and the weaving law of fiber bundle are still not clear, which has a great influence on the physical and mechanical properties of collagen-based materials.

The study of microstructure requires the aid of microscanning technology. The microimage acquisition methods include: microscopic X-ray tomography (MCT), nuclear magnetic resonance (MRI), laser confocal scanning microscopy (CLSM), ultrasonic method and section method. MRI and ultrasound measurements have low resolution (8 ~ 50μm) and are mainly used for medical research and clinical examination. The scale of type I collagen molecule is at the nanometer level, the resolution of these two methods is far from enough. CLSM technology is suitable for transparent materials. For opaque materials, only surface scanning analysis can be performed, so it is not suitable for studying the internal structure of collagen. The method of slicing is to make ultra-thin slices of the sample and observe the fiber section under the microscope5. The resolution depends on the microscope. However, we found that the slices were easily broken when the thickness was less than 3 μm. In addition, the fiber structure was deformed and the image was distorted due to the cutting pressure of the blade.

The research group improved the traditional slicing method, replaced the paraffin-embedded leather sample with rigid material-epoxy resin, layered and polished the sample, observed and analyzed the fiber section structure under the microscope, and obtained the sequence section image. However, certain deformation will occur after fiber embedding, which will affect the accuracy. Therefore, this study compared and studied the MCT images of embedded and non-embedded samples, and studied the deformation and image error, creating conditions for further study on the braided structure of fiber.

2 Experimental Procedures

Leather fibre was obtained from 2 years old American cowhide wet blue. Conventional beamhouse and tanning processes were used to generate the leather. A pelt was depilated using a caustic treatment, and then the residual keratinaceous material was removed. The pelt was splitted, and relimed using 2% (w chemical/w limed leather for all percent chemical additions unless otherwise stated) lime and 0.2% liming auxiliary LAB. After washing, 0.6% Deliming agent DF(TRUMPLER) and 1.8% Ammonium sulfate was added , which gradually increased the pH to 8.4−8.6. The pickled pelt was then chrome tanned and neutralized by sodium sulfate.

Leather fibre were investigated with MCT on wet blue leather(original fibre) after drying, which were taken from butt area according to the regular sample. 2357 imagines with 4032×4032 pixels were got, then 1178 imagines with 2016×2016 pixels were obtained from the same piece of leather which was embedded by epoxy resin(embedded fibre) and investigated with MCT.

3 Results

3.1 Imagine Processing

The 2037 MCT section images with 4032×4032 pixels of the wet blue leather (original sample) was compressed into 2016× 2016 pixels, with 1,178 images first. Then the image denoising is processed by MATLAB, and the denoised images are shown as follows:

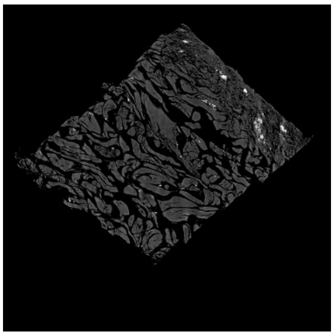

Fig. 1. Image of the wet blue leather (original sample) after denoising.

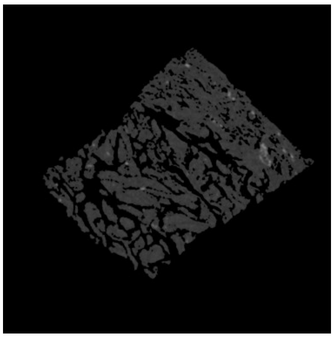

Fig. 2. Image of the embedded leather fibre after denoising.

3.2 Calculation for the Contraction Rate of Embedded Leather Fiber

3.2.1 Contraction rate of embedded leather sample fiber

Because the slice layers of the two types of images are of the same thickness, the same resolution, and the actual size of each pixel is the same, a simple way to compare the volume of the two types of leather sample fibers is to compare the total number of pixels occupied by the two types of fibers.Thus defined the calculation formula as follows:

$$P = \frac{A-B}{A} \times 100\% = \frac{\sum a - \sum b}{\sum a} \times 100\% = \left(1 - \frac{\sum b}{\sum a}\right) \times 100\%$$

P: contraction rate of embedded skin sample

A: volume of in situ MCT slice image

B: volume of embedded MCT slice image

a: pixels of in situ MCT slice image

b: pixels of embedded MCT slice image

The total pixel amount of in situ compressed 1178 MCT slice images of 2016×2016 pixels and embedded 1178 MCT slice images of 2016× 2016 pixels were calculated by MATLAB software as follows:

Total pixels of in situ MCT slice image: 1.0070×10^9 pixels.
Total pixels of embedded MCT slice image: 0.9271×10^9 pixels.

$$P = \left(1 - \frac{0.9271\times10^9}{1.0070\times10^9}\right) \times 100\% = 7.93\%$$

3.2.2 Contraction rate of "Panel"

The "panel" is obtained by filling the interior of the compressed 1178 in-situ MCT slice images of 2016×2016 pixels and the embedded 1178 MCT slice images of 2016×2016 pixels with MATLAB software, as shown in the following figure:

Fig. 3. In-situ MCT slice image "panel" after denoising.

Fig. 4. Embedded MCT slice image "panel" after denoising.

The total number of pixels in the image "panel" is calculated as follows:

Total number of pixels of in-situ MCT slice images "panel": 1.4663×10^9 pixels.
Total number of pixels of embedded MCT slice image "panel": 1.3068×10^9 pixels.

$$Px = \left(1 - \frac{1.3068\times10^9}{1.4663\times10^9}\right)\times100\% = 10.88\%$$

Px: contraction rate of "panel"

3.3 Discussion

3.3.1 Contraction rate of the embedded skin sample

The fiber contraction rate of the embedded skin sample calculated by the above method is 7.93%, and the overall "panel" contraction rate of the embedded skin sample is 10.88%.This indicates that the embedded skin sample has significant shrinkage.

The reason might be the reduction of interval space among the fibre filled with epoxy resin, otherwise the conglutination of fibre caused by the evaporation of solvent(acetone used in embedding) in the course of the resin solidifying. Likewise, it can be the adhesion of the tiny fibre with the larger fibre that will diminish the area calculated. The factors will be studied further on embedding to achieve a method with minimum deformation on cross image of fibre.

3.3.2 Deviation

Due to the strong noise of the embedded leather sample and the difficulty in controlling the denoising scale, different denoising methods will produce different effects. In other words, there may be two problems of insufficient or excessive denoising. This will result in a deviation between the calculated expansion rate of the embedded leather sample and the expansion rate of the "panel" of the embedded leather sample. This needs further analysis.

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