Microstructures of Collagen-Hyaluronic Acid Hydrogels by Two-Photon Fluorescence Microscopy

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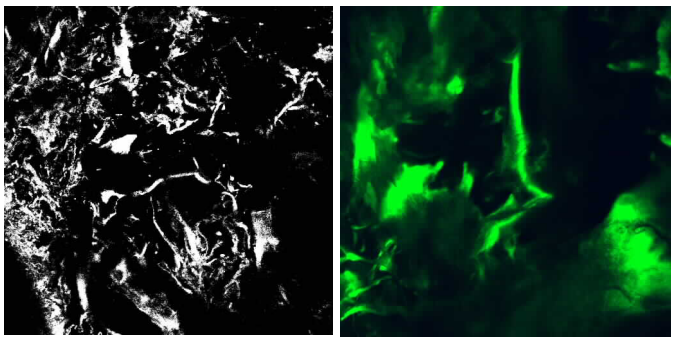
Introduction: Scanning electron microscopy (SEM) and histological techniques have often been used to determine the microstructures of collagen-based hydrogels [1]. However, these techniques entail cross-sectioning, dehydration and/or fixation procedures that inevitably affect the morphology of a hydrogel. Confocal fluorescence microscopy has been successfully used to characterize hydrogel structures [2]. Confocal fluorescence microscopy is of advantage to SEM because the only sample preparation required is the conjugation of a fluorescent dye to the polymer segments in a hydrogel.
Two-photon fluorescence microscopy (TPFM) is an excellent method for cell or tissue imaging because of its inherent 3D resolution and long penetration depth [3]. In addition, this method provides biochemical information about cells or tissues and causes minimal photodamage [3]. In this paper, we describe an application of TPFM to the characterization of the microstructures of collagen-hyaluronic acid (collagen-HA) hydrogels.
Experimental[#]: Collagen-HA hydrogels were fabricated and crosslinked by EDC (Sigma Chemical, St. Louis, MO) as described in our previous paper [4]. For staining the hydrogels, 500 μL of a sodium bicarbonate buffer solution (0.1 mol/L) was added to each hydrogel. 50 μL of an Alexa 430 solution in dimethylsulfoxide (1 % mass fraction Alexa 430, Molecular Probes) was slowly added to the hydrogel immersed in the buffer solution, which was stirred vigorously during the addition. The reaction mixture was continuously stirred for 1 h at 4 °C. The hydrogel was rinsed several times with deionized water and then immersed in α-MEM (α-Minimal Essential Medium, BioWhittaker) for 24 h.
In this study, a two-photon fluorescence microscope (BioRad) was used to characterize the microstructures of two collagen-HA hydrogels, recovered from cell cultures studies that were carried out under similar conditions. The two hydrogels, not denuded of cells, underwent different chemical procedures before their microstructures were examined by TPFM. One hydrogel (untreated hydrogel) was rinsed twice with DMEM (Dulbecco's modification of Eagle's Minimal Essential Medium, buffered with HEPES). The other hydrogel (chemically treated hydrogel) was immersed for 16 min at 37 °C in 3.7 % mass fraction formaldehyde in a cytoskeleton-stabilization buffer at 37 °C [CS: 0.1 mol/L Pipes and 1 mmol/L EGTA in an aqueous solution containing 4 % mass fraction polyethylene glycol 8000 (all from Sigma), pH 6.9]. The hydrogel was then extracted for 9 min in 0.5 % volume fraction triton X-100 (Sigma) in CS, rinsed 3 times in CS, and quenched in freshly prepared 0.15 mol/L glycine in PBS for 10 min at 20 °C. Then, hydrogel was washed twice in phosphate-buffered saline (PBS; without calcium and magnesium chloride), and once in a mounting medium [1:1 glycerol:PBS with 100 mg/mL Dabco (1,4-diazabicyclo[2.2.2]octane; Aldrich)].
Results: The micrographs shown below (the height of each micrograph represents 750 μm) are the images of the optical sections of the untreated hydrogel (right panel) and the chemically treated hydrogel (left panel), each at the depth of 70 μm from the surface. It is worth noting that while the sectional images of both hydrogels exhibit complicated, porous, and network-like microstructures, the image for the chemically treated hydrogel shows a more compact microstructure with smaller pores than the image for the untreated hydrogel. The present study has shown that two-photon fluorescence microscopy is capable of detecting differences in the microstructures that resulted from different treatments of hydrogels. Quantitative image analysis is underway to characterize the microstructures of the hydrogels. It is expected that two-photon fluorescence microscopy will provide microstructural characteristics of scaffolds for tissue engineering.



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