Evaluation of collagen crosslinking using second harmonic generation and coherent anti-Stoles Raman scattering microscopy

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ABSTRACT

Nonlinear optical microscopy has become a promising tool to identify the structure and function of biotissues in a label-free and noninvasive manner [1]. It leads to the applications of examining and monitoring bioengineered materials during the fabrication processes. To provide a comprehensive insight into the collagen structure at molecular and macromolecular scales, the contrast mechanisms of second harmonic generation (SHG) and coherent anti-Stoles Raman scattering (CARS) are used, which are responsible for structural and chemical specificity respectively, to characterize multiple collagen scaffolds with different crosslinking treatments. In this work, the SHG images showing specific structure patterns organized by collagen molecules were used to analyze the molecular orientation distribution, structure anisotropy, fast Fourier transform spectrum, and coherency map [2]. Interestingly, the result of coherency map is correlated with the compressive property of the crosslinked samples, which was confirmed by a tensile strength tester. On the other hand, the degree of integrity of collagen triple helix was estimated with comparative analyses of SHG signal and relative CARS signal of amide III band at 1240 cm^{-1} to δCH2 band at 1450 cm^{-1} [3] of these crosslinked samples. Both results showed that the measured values can be categorized into three groups: raw materials, scaffolds with dehydrothermal treatments and scaffolds with chemical crosslinking methods. It manifests that the raw materials preserve the integrity of collagen triple helix, which assists SHG emission and higher absorption ratio of A_{III}/A_{1450}, as compared to these crosslinked scaffolds that experienced a partial denaturation of the molecular structure. Consequently, SHG/CARS microscopy provides information regarding the variations in macromolecular and molecular structures during a crosslinking process, which can then be served as unique biosignatures to identify the crosslinking status.

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