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Wavelength-Dependent Conformational Changes of Collagen in Mid-IR Ablation M. SHANE HUTSON, YAOWU XIAO, Dept of Physics and Astronomy, Vanderbilt University and VIIBRE - Vanderbilt Institute for Integrative Biosystem Research and Education, MINGSHENG GUO, Dept of Physics, Fisk University —
Single pulses of the Mark-III free electron laser have been used to ablate porcine corneas at a fluence of 240 J/cm^2 and wavelengths of 2.77 and 6.45 μm. As previously characterized, the non-volatile ablation debris shows evidence of wavelength-dependent collagen fragmentation. We have measured micro-Raman spectra of the debris and the ablation crater to determine if any wavelength-dependent conformational changes have taken place. Comparison of the spectra from two different wavelengths shows that a 938 cm^{-1} Raman band – assignable to the peptide $C_{C=O}$-C_α stretch of collagen – loses substantial intensity during 6.45-μm ablation, but not in 2.77-μm ablation. This intensity decrease may be associated with a reduction of collagen triple-helix structure. Other spectral techniques yield mixed results; signatures for the loss of triple-helix structure are evident in UV-CD and some aspects of ^{13}C-NMR spectra, but not in FTIR spectra. Raman measurements on thermally-treated corneal slices display similar changes at high temperatures, suggesting that higher protein temperatures are reached during ablation at 6.45 μm when compared to 2.77 μm. These observations suggest that any pre-vaporization loss of protein structural integrity includes not only collagen fragmentation, but also a loss of collagen triple-helix structure.

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