Multiphoton Image Parameters Predict Mechanical Stiffness of Cellularized Collagen Gels

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Statement of Purpose: Cellularized collagen gels are widely used in vitro models of connective tissue, but the microstructure-mechanics relationship of these gels remains poorly understood. Multiphoton microscopy (MPM) of second harmonic generation (SHG) by fibrillar collagen reveals fiber morphology and network characteristics, whereas two-photon fluorescence (TPF) signal can be used to image collagen crosslinks. SHG signal scales with the square of collagen harmonophore concentration; TPF signal scales directly with fluorophore concentration. Hypothetically, quantification of these two optical signals' intensities, and of collagen network features using image analysis of multiphoton images, may allow prediction of bulk tissue mechanics.

Methods: Normal human lung fibroblasts (Cambrex) were expanded in vitro in DMEM (Sigma) containing 10% FBS, bFGF, and IGF (cytokines from Lonza). Upon confluence, fibroblasts were mixed with acid-soluble rat tail type I collagen (BD Biosciences), which was allowed to polymerize at pH 6.5 or 8.5 for one hour, to create coarse and fine fiber networks, respectively (Raub, CB, *et al.*Biophys J. 2008; 94(6):2361-73). Following polymerization, tissues were maintained in floating culture in standard incubation conditions for up to two weeks. In some cases media was supplemented every other day with 2 ng/ml TGFβ-2, 35 pg/ml PGE-2, or 1 μM GM6001. Gels were removed periodically from floating culture, imaged with a multiphoton microscope (LSM 510Meta, Zeiss) and mechanically tested with a uniaxial material tester (Biometra, MTS Corporation). MPM was performed at wavelengths of 780 nm; SHG signal was collected at 385-395 nm; TPF signal was collected at 500-550 nm. A ramp compression test was performed on the gels with a 1.3 mm diameter platen, at a strain rate of 0.05 mm/s, to 10% strain, and the modulus was recorded from the stress/strain slope. Single and multiple linear regression was performed on the mechanical data regressed on several MPM image parameters, including SHG signal intensity, TPF signal intensity (normalized by SHG signal), and average mesh size, calculated in μm using particle analysis on thresholded SHG images. Prior to imaging and mechanical testing, gel volume was estimated by caliper measurements of gel height and diameter, and collagen concentration was calculated from these dimensions. A Sircol assay (Accurate Chemical and Scientific Corp.) demonstrated constant collagen mass content in the gels regardless of extent of gel contraction or addition of soluble factors.

Results: After 1 week in the presence of TGFβ-2 or FBS, coarse-structured cellularized collagen gels contracted to 11.4±2.6% and 14.5±1.5% of their original volume, respectively. Gels treated with GM6001 and PGE-2 contracted to 28.5±2.6% and 59.2±11.5% of their original volume, respectively. Fine-structured gels contracted less than coarse-structured gels, by 5%, 15%, 55%, and 27% for TGFβ-2, FBS, GM6001, and PGE-2 treated gels, respectively. DQ collagen imaging revealed little collagen degradation above acellular controls. Regardless of coarse or fine microstructure, collagen concentration explains compressive modulus in these uncrosslinked gels very well (linear regression; R^2=0.82). Glutaraldehyde crosslinking increased the TPF signal in a collagen concentration-dependent manner (slope of linear regression, 11.5 arbitrary fluorescence units/mg collagen/ml; R^2=0.98), leaving SHG signal unaffected. When comparing uncrosslinked and crosslinked gels, collagen concentration alone is a poor predictor of collagen gel bulk compressive modulus (R^2=0.52, Figure 1, top panel). Multiple linear regression of mechanical data on SHG intensity and the TPF/SHG intensity ratio, following log transformation, reveals power law exponents of these optical parameters of 1.3 and 0.62, respectively, which allow good correlation of mechanics data with optical parameters (R^2=0.98, Figure 1, bottom panel).

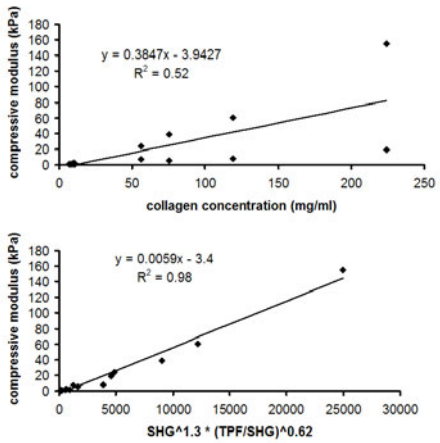

Figure 1.
Collagen concentration fails to capture variation in compressive modulus due to crosslinking (top panel). The SHG signal and TPF/SHG ratio capture concentrations of collagen and collagen crosslinks, and together explain mechanical data with a power-law relationship (bottom panel).

Conclusions: TGFβ-2 and PGE-2, and the metalloproteinase inhibitor GM6001 modulate cell-mediated collagen gel contraction, but collagen concentration alone explains gel compressive modulus, regardless of other biological effects of these soluble factors and of fine or coarse fiber microstructure. Incorporation of crosslinks into the collagen gel reduce the ability of collagen concentration to explain gel mechanics, but the SHG signal intensity and TPF/SHG ratio scale with collagen protein and crosslink concentration, respectively, and together explain variations in compressive modulus of cellularized collagen gels.