Statement of Purpose: Type I collagen is both the most prevalent protein in the human body and an ideal candidate biomaterial for tissue engineering. Collagen molecules may be isolated from various tissues using enzymatic and non-enzymatic techniques; however, they display different self-assembly capabilities—the ability to transition into fibrillar matrices at physiologic conditions. The stress-strain curve of self-assembling collagens, whether formed in vitro or in vivo, transitions from linear to exponential at a critical strain value which is determined in part by the overall network branching and connectivity [1]. This nonlinear elasticity, besides contributing to the mechanical behavior of the material, has been proposed to influence stem cell fate independent of linear stiffness [2].

To date, the complete molecular mechanisms of collagen self-assembly have yet to be elucidated, and most studies alter temperature or apply exogenous crosslinkers to control critical strain of self-assembling collagens. Recently, we described the isolation and purification of collagen oligomers from porcine dermis, which retain natural intermolecular crosslinks [3]. Turbidimetric assays and microscopy showed these oligomers undergo rapid self-assembly into highly branched networks. Mechanical analysis revealed higher shear and elastic moduli than traditional monomeric collagens [3-6]. Here we extend these findings by performing detailed molecular and biophysical analyses, comparing oligomeric collagen to conventional atelocollagen and telocollagen formulations.

Methods: Collagen oligomers were extracted as previously described [3]. Pepsin-solubilized bovine collagen (atelocollagen; Advanced Biomatrix) and acid-solubilized rat tail collagen (telocollagen; Corning or Advanced Biomatrix) were used as commercial comparisons. Dynamic light scattering experiments were conducted with a ZetaSizer ZS90 (Malvern Instruments). Measurements were acquired at a 90° scattering angle using a glass scattering cuvette with a 10mm path length. Rheological experiments were conducted using an AR-2000 rheometer (TA Instruments). A 40mm steel parallel plate geometry was used to apply a logarithmic strain sweep at a fixed frequency of 1.0 Hz from γ = 0.001-2.0 or until material failure. Self-assembly capacity was compared using an established polymerization competence assay [7]. All measurements were acquired in at least triplicate. Minitab was used to perform statistical comparisons with p≤0.05 considered significant.

Results: Dynamic light scattering revealed multimodal distributions with a dramatic shift towards larger molecules for oligomeric collagen compared to atelocollagen and telocollagen preparations (Figure 1A). Unlike oligomer and telocollagen, atelocollagen preparations showed a highly variable and broader molecular size pattern. Significantly less residual collagen was detected in the supernatant of self-assembled matrices prepared with oligomeric collagen or telocollagen than from atelocollagen, suggesting collagen without telopeptides was either polymerization deficient or produced less stable fibrils (Figure 1B). Comparison of stress-strain curves for the various formulations at matched concentrations showed that oligomers displayed the highest yield stress (Figure 1C). Plotting the differential shear modulus (K=dσ/dγ) against oscillatory stress for a concentration range further demonstrated fundamental differences in the nonlinear elastic response that are fibril-density independent (Figure 1D). Independent of concentration, collagen oligomers transition to the exponential stress regime significantly earlier than telocollagen and atelocollagen. The critical strain at onset of stiffening (10% increase in G‘) for oligomeric collagen, atelocollagen, and telocollagen were 0.041±0.01, 0.064±0.01, and 0.185±0.02.

Conclusions: Oligomeric collagen, with its natural intermolecular crosslinks, represents a highly pure collagen composition with distinct self-assembly capacity, yielding more highly stable and interconnected fibril microstructures compared to conventional atelocollagen and telocollagen preparations. Future studies will be conducted to evaluate the effects of differences in strain-stiffening on cell phenotype.

References: