Dynamic Observation of Collagen Assembly and Degradation

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Statement of Purpose: Fibrillar collagens are the principal load-bearing proteins in animals. Their specific organization is critical to the function of load-bearing structures including bone, tendon, cartilage and ligament. Organization of collagen arises during development, growth and remodeling but collagen turnover and turnover kinetics at level of the fibrils are not well-understood. It has been shown that the collagen turnover rate in different tissues varies between 50 to 200 days in humans and between 10 to 100 days in mouse and rabbit [1]. Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases involved in the degradation of the extracellular matrix, including collagen, in humans. It has been suggested that these enzymes play important role in cells migration and differentiation [2]. Despite the importance of these enzymes function in nature, little is known, quantitatively and qualitatively, about them and all the aspects of their functions, particularly the rate at which they degrade their target. Collagenase, which is more readily available, is a broad spectrum collagen cleavage molecule utilized by invading bacteria and has been more thoroughly investigated. The objective of this investigation is to develop a low-volume method and system to facilitate direct observation of collagen self-assembly and collagen catalysis *in vitro*.

Methods: Cold, neutralized, acid-soluble bovine collagen monomers (3.0 mg/mL, Inamed Biomaterials, Fremont, CA) in PBS were injected through an in-line solution heater (SC-20, Hamden, CT) into an environmentally-controlled glass microfluidics chamber (FCS2 Chamber, Bioptechs, Butler, PA) mounted on the stage of an inverted microscope (Nikon TE2000-U with PFS®, Melville, NY). The solution was warmed to 37°C prior to entry into the chamber and maintained at that temperature throughout the experiment. Subsequent to the injection of the collagen, the nucleation of collagen fibrils on the surface of the glass bottom of the chamber was followed dynamically (images every 5 sec) with Differential Interference Contrast imaging (DIC - 60x objective; 1.4 NA; 1.5x). After 10 minutes, activated solution of 0.05 mM collagenase (Cat# C0130, Sigma-Aldrich, St. Louis, MO) was injected at 37°C into the chamber and the dynamics of collagen degradation was observed and recorded (images every 5 sec).

Results/Discussion: The dynamic DIC images capture the nucleation and axial growth of collagen, and show that nucleation begins at approximately 150 seconds with an axial growth rate of approximately 8.0 microns/min (which was allowed to progress for 10 minutes). The time for complete digestion of the self-assembled fibrils given 0.05 mM collagenase exposure was ~180 seconds, one-third the time it takes for the same mass of collagen fibrils to assemble.

Conclusions: DIC imaging in combination with a temperature-controlled microfluidics chamber is an effective system which can be used to observe the kinetics of collagen self-assembly and degradation due to the presence of collagenase. It has already demonstrated that the time-to-degradation of collagen in the presence of bacterial collagenase is one-third of the time-to-assembly of collagen. Given the small volume and versatility of the chamber it is possible (economically) to begin similar experiments designed to examine the rate of MMP-1 degradation of self-assembled collagen *in vitro*. The system will ultimately allow us to examine how different fibril morphology (depending on assembly conditions) affect the rate of MMP-1 cleavage.

References:

[1] Gineyts E. Biochem J. 2000;345:481-485.
[2] Enciso JM. Pediatrics 2005;116:228-230.