Shankar
Senior Division Medicine & Health
Proteomic Characterization Of Extracellular Matrix Using Sonication-Assisted Digestion
The proteomic composition of the extracellular matrix (ECM) influences the growth and motility of cancer cells. The identification of differences between healthy and cancerous ECM can provide a list of candidate biomolecules that can be validated as tumor suppressors or promoters. However, the traditional approach of overnight protein digestion using enzymes (a key step in ECM analysis) does not work well for ECM samples. A novel set of methods are required to address the digestive resistant properties of ECM. Sonication, the application of ultrasound energy, has proven to accelerate protein digestion in cellular protein samples. It is hypothesized that sonication will efficiently digest ECM samples. Four variables were evaluated to maximize the efficiency of digestion: sonication time, intensity, sample temperature and reagent concentrations. Experimental results showed that sonication considerably improved ECM digestion. However, the 1-D gel electrophoresis showed that for sonication-assisted digestion, degradation occurred with many protein samples, possibly due to the liquid cavitation event. Subsequent experimentation using free radical scavengers to counter ECM protein fragmentation showed that sonication efficiently digested proteins while preventing degradation. This research makes three important contributions: a) previously, only approximately 30 proteins had been identified in Matrigel (due to the difficulty of analyzing ECM samples) … this research has identified 200+ proteins, b) it demonstrates the novel application of sonication to efficiently digest ECM samples, and c) it accelerates protein digestion from overnight to a few minutes. These results are expected to significantly enhance the ability of researchers to explore the impact of ECM on cancer.