Preparation and Characterization of Collagen Substrates for Protein Interactions Studies

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Statement of Purpose: Collagen is an important extracellular matrix (ECM) protein in the body, making up 25-35% of the body's total protein. In vivo, collagen forms a hierarchical fibril structure, with its peptide chain containing a high concentration of glycine, proline, and hydroxyproline. In addition to its structural and mechanical properties, collagen interacts with other ECM molecules, clotting proteins, cell adhesion proteins, and growth factors. Collagen is also important for tissue engineering applications, where it can provide structural stability, support cell attachment and signaling, and promote angiogenesis. Since collagen plays such an important biological role, it is commonly used as a substrate for *in vitro* assays, but further characterization is needed to understand how variations in the experimental conditions and sample preparation methods affect the final structure and composition of the collagen substrates.

Methods: Human placenta type III collagen was purchased from Sigma (Source 1) and Abcam (Source 2) and used without further purification. Unless otherwise noted, substrates were tissue culture treated polystyrene dishes (TCPS, Corning). Collagen was adsorbed from degassed phosphate buffered saline (PBS) (137mM NaCl, 2.7mM KCl, 10mM phosphate) at pH 6.5 or 8.0. X-ray photoelectron spectroscopy (XPS) data were collected on a Surface Science Instruments S-Probe instrument with monochromatized Al K_α x-rays. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) data were acquired on an IONTOF 5-100 instrument using a Bi^{3+} primary ion source under static conditions. The sum frequency generation (SFG) vibrational spectra were obtained with an EKSPLA picosecond SFG system by overlapping visible and tunable IR laser pulses in time and space.

Results: Collagen adsorbed from 100µg/ml solutions at pH 6.5 resulted in a higher XPS nitrogen signal from source 1 (~11 atomic %) compared to source 2 (~7 atomic %). An XPS nitrogen content of 11 atomic % is consistent with a monolayer of protein adsorbed onto the TCPS surface. ToF-SIMS results were consistent with the XPS results. The sum of intensities from all amino acid peaks normalized to the total secondary ion intensity showed that source 2 resulted in more collagen adsorption onto the TCPS surface. Also, the intensities of peaks associated with the TCPS substrate are attenuated more by adsorption of Source 2 collagen than by adsorption of Source 1 collagen. The XPS and ToF-SIMS results suggests the difference between these two collagen surfaces is primarily due to a difference in surface coverage and not thickness of the fibrils. ToF-SIMS indicated that both collagen sources had contained similar amounts of hydroxyproline (10-15% of the ToF-SIMS amino acid signals were from the hydroxproline peak at m/z = 86.067).

Principle component analysis (PCA) processing of the ToF-SIMS data was used to investigate the interactions of the A1 domain of von Willebrand Factor with the collagen surfaces. PCA easily differentiated between pure collagen covered surfaces and pure A1 covered surfaces, based on the different amino acid compositions of these two proteins. For samples that have been sequentially exposed to collagen from Source 1 and then A1, the PCA scores are intermediate between the single component collagen and A1 scores. As the concentration of A1solution is increased from 10 to 100 µg/ml, the surface changes from mostly collagen-like to mostly A1-like. This could be due to either different amounts of A1 binding to collagen or A1 adsorbing onto the bare TCPS surface that is present from the lower surface coverage resulting from source 1 collagen adsorption. A different trend is observed for A1 interactions with the Source 2 collagen substrate. Varying A1 solution concentration results in samples with similar PC1 scores that are close to the collagen-only sample. This could be due to either the Source 2 collagen surface having a lower A1 binding capacity compared to Source 1 collagen or A1 preferentially adsorbing onto the small amount of TCPS substrate exposed on these samples. SFG spectra of the C-H and amide I bands clearly shows differences in the surface structure of collagen adsorbed from pH 6.5 versus pH 8.0 solutions. Collagen adsorbed at pH 8.0 shows significantly higher SFG amide I signals than collagen adsorbed at pH 6.5 (Fig. 1), demonstrating that collagen adsorbed from a pH 6.5 solution contains little helical content. In contrast, collagen adsorbed from a solution of pH 8.0 has an ordered peptide backbone and retains helical structure on the surface. XPS shows significantly more collagen is adsorbed at pH 6.5 compared to pH 8.0 (11 vs. 2 atomic % nitrogen), so the increased ordering of the helical backbone at pH 8.0 is not due to an increased amount of protein on the surface.

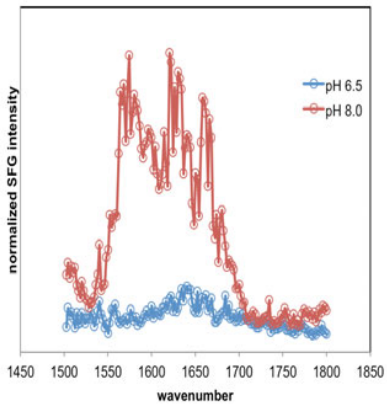

Figure 1. SFG Amide I collagen spectra.

Conclusions: The results of this study demonstrate that experimental conditions and collagen source can greatly affect the nature of collagen substrates. Thus, thorough, multi-technique characterization is necessary to define the structure and composition of collagen substrates.

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