Human Fetuin-A Treatment for Demineralization of Arteriosclerosis

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Purpose: Premature death from cardiovascular disease is especially high in patients with chronic kidney disease. Studies have shown that deficiencies in serum levels of Fetuin-A inversely correlate with the extent of vascular calcification and are associated with increased cardiovascular mortality in at-risk patient groups [2]. Human Fetuin-A is a serum glycoprotein and a well-known regulator of bone metabolism. It is hypothesized that Fetuin-A functions as a chaperone protein to form a calciprotein particle (CPP), where a protein coating develops around nascent apatite mineral inhibiting further growth and facilitating in its removal from cells and soft tissues [2]. Prior to the development of a delivery vehicle to allow for the targeted release of Fetuin-A to calcified arteries, the binding interactions of Fetuin-A with amorphous calcium carbonate (ACC) nanoparticles was examined. This was done in order to understand specific functional group interactions between the protein and calcium, examine the stabilization effect of Fetiun-A on pathological mineral growth and aggregation, and to develop experimental methods to monitor Fetuin-A concentrations and binding interactions. A slightly acidic local environment, characteristic of arteriosclerosis, was utilized for this study to stimulate protein release at a vascular calcification site.

Methods: Human Fetuin-A (Sigma-Aldrich, St. Louis, MO), ACC nanoparticles (SkySpring Nanomaterials, Houston, TX), and a complex of human Fetuin-A with ACC nanoparticles (Fetuin-A/ACC) were prepared and analyzed in deionized water or Dulbecco's Modified Eagle's Medium (DMEM) (Fischer Scientific, Waltham, MA), a body fluid simulant. Molecular structure, surface chemistry, and binding of Fetuin-A/ACC was investigated with attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy equipped with a diamond/zinc-selenide crystal (Nicolet 6700) and X-ray photoelectron spectroscopy (XPS) with a 1600 Phi XPS system (Physical Electronics). Fetuin-A and ACC nanoparticle binding interactions were also characterized using a Shimadzu 2550 UV/Vis spectrophotometer.

Results: To the authors' knowledge, this is the first reporting of ATR-TIR spectroscopy of the Fetuin-A protein. The FTIR spectrum reveals amide I and II peptide bonds at ~1650 and 1550 cm^{-1}, respectively, characteristic of protein peptides (Figure 1). Samples of Fetuin-A complexed with ACC nanoparticles (Fetuin-A/ACC) did not show the amide II absorption band. UV/Vis spectral analysis showed protein unfolding transitions as measured by the increase in absorbance at 287 and 291 nm for tyrosine and tryptophan, respectively. This increase in absorbance is a function of ACC NP concentration. Elemental surface composition using XPS of the ACC nanoparticles was 32.83% C, 48.81% O, and 18.36% Ca as compared with a 20% C, 60% O, and 20% Ca theoretical ACC atomic concentration. Further surface analysis of Fetuin-A/ACC complex revealed atomic mass percentages of 61.43 % C, 31.30% O, and 7.27 % N, showing no calcium content, the presence of nitrogen, and a larger carbon content which are characteristics of various glycoproteins [3].

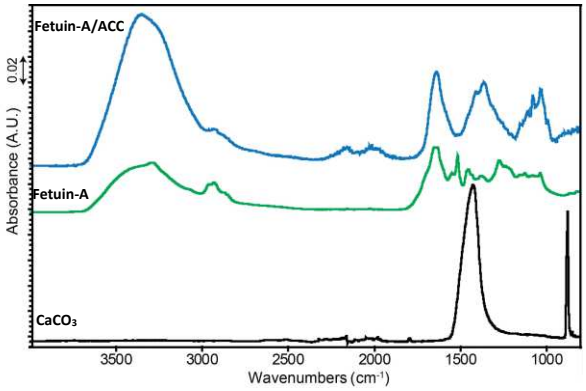

Figure 1: Representative FTIR spectra for $CaCO_3$, Fetuin-A, and Fetuin-A/ACC.

Conclusions: The lack of Ca detected by XPS for the Fetuin-A/ACC samples indicates Fetuin-A has bound with the $CaCO_3$ nanoparticles forming a protein coat during calciprotein particle (CPP) development and subsequent mineral stabilization. ATR-FTIR analysis of the Fetuin-A/ACC complex showed a loss of the Amide II peak, indicating protein absorption through the amide group onto the nanoparticles. The observed UV/Vis shifts suggest that specific amino acid residues, tyrosine and tryptophan, participate in the ability of Fetuin-A to stabilize ACC growth and aggregation. These results support the hypothesis that Fetuin-A forms a protein coat around calcium mineral in order to stabilize and inhibit further growth.

Future Work: Methods to modify the Fetuin-A protein via biochemical methods (i.e. amination) will be examined in order to enhance Fetuin-A binding without compromising the protein's natural ability for cellular uptake and clearance of CPPs. In addition, the synthesis of biocompatible polymersomes for protein encapsulation and targeted delivery near vascular calcification sites will be a focus area.

References:

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