Fetuin-A Therapy: A New Approach for the Treatment of Vascular Calcification

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INTRODUCTION

The leading cause of death among patients with chronic kidney disease (CKD) is cardiovascular disease [1]. The development of vascular calcification (VC) in the arteries is one of the main issues that cause the onset of cardiovascular complications leading to increased cardiac morbidity in CKD patients. Arterial medial calcification occurs more exclusively in muscle-type arteries and is most common in patients in end stage renal disease (ESRD) [1]. Also, the rate of arterial medial calcification development in ESRD patients is closely associated with the duration of dialysis treatment and Fetuin-A deficiencies [1]. Fetuin-A is a glycoprotein that functions as a systematic regulator of biomineralization. Studies have shown that Fetuin-A $^{-/-}$ null mice suffer from extensive soft tissue calcification which is accelerated by a mineral rich diet, suggesting that Fetuin-A plays a key role in the inhibition of VC development [2]. We are proposing the development of a Fetuin-A therapy consisting of a pH responsive, biodegradable polymersome that can carry and target the delivery of Fetuin-A directly to sites VC. Fetuin-A would serve as a chaperone to facilitate the removal of calcium phosphates precipitates from the artery walls. In order to progress to the end goal of developing the Fetuin-A therapy, this study focused on characterizing the stabilizing effects of Fetuin-A on suspended $CaCO_3$ nanoparticle growth in a simulated body fluid (SBF) model.

METHODS

Dynamic light scattering (DLS) (ZetaPALS Instrument (Brookhaven Instruments Corporation (BIC)) was used to determine the average hydrodynamic diameter of human Fetuin-A (Sigma-Aldrich, St. Louis, MO), $CaCO_3$ nanoparticles (SkySpring Nanomaterials, Houston, TX), and a combination of human Fetuin-A with $CaCO_3$ nanoparticles in Dulbecco's Modified Eagle's Medium (DMEM) (Fischer Scientific, Waltham, MA) to simulate body fluid. A minimum of 5 DLS measurements were collected for each sample, and averages and standard deviations were reported. Samples containing 100µl Fetuin-A in SBF, 0.1 mg/ml $CaCO_3$ nanoparticles in SBF, and a combination of $CaCO_3$ nanoparticles along with Fetuin-A in SBF were analyzed. Scanning (JEOL JSM-6500 field emission scanning electron microscope (FE-SEM) operated at 5 or 15 keV) and transmission electron microscopy (JEOL 2100 at 200 kV) were used to reveal the surface morphology and ultrastructure of the calcium carbonate nanoparticles and the Fetuin-mineral complexes.

RESULTS

DLS measurements on hydrodynamic size distribution reveal mean effective diameters of $CaCO_3$ nanoparticles in SBF and Fetuin-A protein in SBF to be 424.4 ± 19.65 nm and 6.21 ± 1.19 nm respectively. The addition of 100 µl Fetuin-A to the $CaCO_3$ nanoparticles resulted in mean hydrodynamic diameters in SBF of 452.6 ± 15.3 nm.

Morphological characterization of the calcium carbonate nanoparticles and the fetuin-mineral complex were assessed using TEM, the images reveal a range of large 400-1500nm structures of $CaCO_3$ nanoparticle clusters and a similarly sized double membrane fetuin-mineral complexes. A change in amorphous calcium carbonate nanoparticles from cubic to spherical was observed with SEM upon the addition of nominal amounts of Fetuin-A. The images taken with the TEM and SEM are shown below in Figure 1.

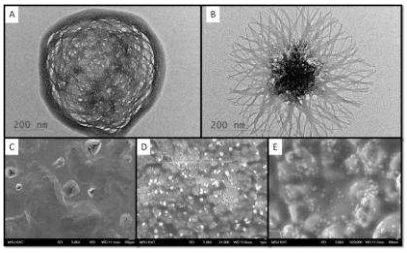

Figure 1: Top row: (A)TEM of unstabilized fetuin-mineral complex, (B) TEM of stabilized fetuin-mineral complex; Bottom row: SEM images of fetuin-mineral complexes (C) sb; 100 µm (D) sb; 1 µm(E) sb;100 nm

CONCLUSION

The results of this study mimic the hypothesized binding mechanism of Fetuin-A to calcium (shell like encapsulation of the mineralized core of $CaCO_3$ that is visualized in Figure 1A) and the stabilizing effects of Fetuin-A on $CaCO_3$ nanoparticle growth and precipitation. From this study, it is shown that even at nominal concentrations, Fetuin-A has a partial encapsulation effect on a portion of the suspended $CaCO_3$ nanoparticles. The low levels of Fetuin-A were unable to stabilize all of the mineral growth which is illustrated by the presence of large >1200 nm structures on the SEM images. Higher concentrations of Fetuin-A would most likely improve the encapsulation and stabilizing effects of Fetuin-A on mineral nuclei.

FUTURE WORK

Future work will focus on biodegradable polymer development for Fetuin-A encapsulation. To understand more about Fetuin-A's ability to stabilize mineralization, the Fetuin-mineral complex uptake and clearance will be analyzed in vitro along with the synergistic effects of Fetuin-A and other mineralization regulators on vascular calcification.

REFERENCES

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