Developing dual-delivery nanogels for treatment of myocardial infarction

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Statement of Purpose: Myocardial infarction (MI) affects approximately 785,000 people each year in the US alone[1]. MI typically occurs as a result of plaque rupture and thrombus formation, which occludes one of the coronary arteries and causes ischemia. Reestablishing blood flow to the ischemic tissue is paramount for treating MI, however, reperfusion injury can contribute to cardiac fibrosis. Here we aim to develop a novel dual-delivery nanogel system that will simultaneous address the need to 1) quickly reestablish blood flow and 2) inhibit cardiac fibrosis following reperfusion injury by first releasing a fibrinolytic protein to lyse the occlusion and then delivering a small molecule cell contractility inhibitor to prevent cardiac fibrosis following reperfusion injury. Here we employ a CoreShell poly(N-isopropylacrylamide) (pNIPAM) nanogel comprised of a highly crosslinked core and a loosely crosslinked shell to achieve temporal control of drug release. We hypothesize that following loading, the larger fibrinoytic drug will be partitioned primarily into the nanogel shell allowing for a burst release, while the small molecule fibrosis inhibitor will efficiently infiltrate the highly cross-linked core and enable sustained release. We expect that loading nanogels with various ratios of small and large molecules will alter the release profiles. In these studies, we aim to determine optimal loading conditions to achieve sustained release of small molecules and burst release of large molecules.

Methods: CoreShell pNIPAM nanogels were synthesized in two precipitation polymerization reactions. Synthesis of the nanogel core incorporates 90% NIPAM and 10% methylenebisacrylamide (BIS) crosslinker to achieve high crosslinking. The nanogel shell synthesis includes 93% NIPAM, 2% BIS crosslinker, and 5% acrylic acid to achieve loose crosslinking. Hydrodynamic diameters of Core and CoreShell nanogels were determined through Nanosight particle tracking and particle area and height were determined through atomic force microscopy (AFM). To examine release characteristics when loading nanogels with various ratios of small and large molecules, green and red fluorescently labeled dextran (Fluoresein isothiocyanate (FITC)-20kDa dextran and Rhodamine B (RhoB)-6kDa dextran) were loaded into nanogels through a one-step "breathing in" strategy. Particles were lyophilized and then hydrated at 20 mg/mL for 24 hours with one of five labelled dextran solutions: 225µg/mL RhoB dextran (Red only), 225µg/mL FITC dextran (Green only), 225µg/mL RhoB dextran + 225µg/mL FITC dextran (1:1 Red:Green), 225µg/mL RhoB dextran + 112.5µg/mL FITC dextran (2:1 Red:Green), and 112.5µg/mL RhoB dextran + 225µg/mL FITC dextran (1:2 Red:Green). Following loading, particles were centrifuged at 21,100xg for 30 minutes and the release profiles were determined by analyzing fluorescence in the supernatant at various time points (1, 2, 6, 18, 44, and 168 hours).

Results: The hydrodynamic diameter of CoreShell particles was 244 ± 36 nm, compared to Core particles at 162 ± 30 nm, demonstrating successful shell addition. AFM analysis (Figure 1A-D) demonstrate increased particle diameter from 181 ± 22 nm to 238 ± 30 nm and increased height from 20 ± 3 nm to 29 ± 6 nm after Shell addition. Figure 1E highlights the first 25 hours of release of red and green dextran for each loading condition. When loaded with 2:1 Red:Green, the nanogels show a more sustained release of smaller 6kDa molecules compared to other conditions. Burst release of the larger 20kDa dextran molecules remains consistent throughout the loading conditions with the exception of 1:2 Red:Green, with decreased initial burst concentration. All loading conditions show essentially complete release of the larger molecules within 6 hours. Comparatively, 2:1 Red:Green conditions still showed an average release of 19µg/mL of smaller 6kDa molecules at 6 hours and remained at detectable levels at 168 hours.

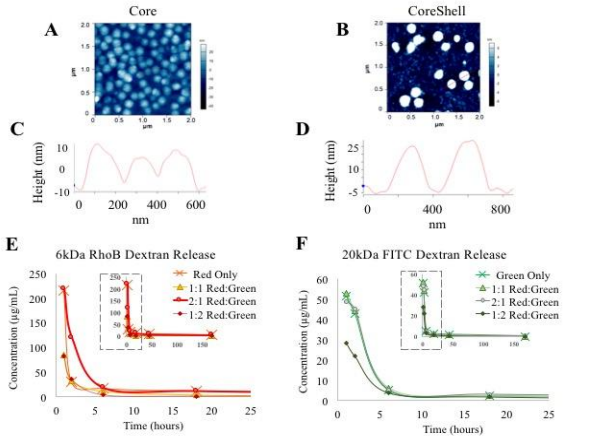

Figure 1. AFM images (A, B) and corresponding particle sizes for Core and CoreShell nanogels (C, D). Release profiles of small 6kDa (E) and large 20kDa (F) molecules.

Conclusions: CoreShell synthesis produced nanogels with differential crosslinking densities in the core and shell that allowed molecules of different sizes to be partitioned within the nanogel to facilitate fine temporal control of the dual-delivery system. Release dynamics are dependent on loading ratios of small and large molecules, with 2:1 Red:Green loading solutions showing the most sustained release of small molecules. Future studies will incorporate fibrinolytic proteins and small molecule fibrosis inhibitors into the nanogels and then analyze the effects of drug-loaded nanogels on fibrin clot lysis and inhibition of fibrosis associated phenotypes in vitro.

References:

[1] Mozaffarian D. et al. *Circulation*. 2015;131:e29-e322.