Amphiphilic Nanoparticles as Molecular Therapeutics for Atherosclerosis

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Statement of Purpose: Cardiovascular disease (CVD) accounts for ~50% of all deaths in the West, costing $440 billion annually[1]. Atherosclerosis, the major trigger for CVD, is defined by persistent inflammation and the buildup of lipid-rich plaques in arterial walls[1]. Atherosclerosis develops over time as a result of a high concentration of oxidized low density lipoprotein (oxLDL) deposited within the blood vessel walls and taken up uncontrollably by macrophages, leading to the formation of foam cells (the primary constituent of atherosclerotic plaques)[1]. The plaques can then become vulnerable and break off the membrane causing myocardial infarction or stroke[1]. A wide array of therapies has been developed to treat atherosclerosis, all of which have inherent limitations, primarily because they exhibit various off-target effects and fail to undo years of accumulated damage of plaque buildup[1]. In an attempt to address these limitations, the Moghe laboratory at Rutgers has advanced kinetically assembled amphiphilic macromolecule (AM) nanoparticles (NPs) that are capable of inhibiting the uptake of oxLDL in human macrophages[2], thus, preventing foam cell formation and plaque development[2]. The focus of this work is to elucidate the mechanism(s) of oxLDL inhibition for the design and development of future NP-based therapeutics and identify the most promising therapeutic leads.

Methods: The AMs (termed 1cM, 1nm, 2cbM, and meso, 1cT) and the hydrophobic NP core material (M12), synthesized by modifying mucic acid with lauroyl chloride, were prepared as previously described[3]. Kinetically assembled AM NPs were fabricated via flash nanoprecipitation (Figure 1B)[2]. Human peripheral blood mononuclear cells (PBMC) were cultured for 7 days in RPMI 1640 supplemented with 10% FBS, and 50 ng/mL M-CSF for differentiation into macrophages. Macrophages were then transferred into 48 well plates, allowed rest for 24 h, and then treated with NPs ([AM] = 1 x 10^{-5} M) + DiO oxLDL (5 µg/mL) for 24 h. Nanoparticle and oxLDL uptake were evaluated via microscopy and flow cytometry, cell marker expression by flow cytometry, and gene expression by q-RT-PCR.

Results: A library of nanoparticle chemistries, 1cM, 1nm, 2cbM, and meso 1cT, were fabricated (Figure 1A). All NP chemistries were capable of inhibiting the uptake of oxLDL to varying degrees and become readily associated with the macrophages through internalization and/or surface binding, Figure 1C. In this study, the top NP candidates exhibited a two-pronged mechanism for preventing oxLDL uptake, including direct binding interactions with scavenger receptor A (SR-A), and the down-regulation of gene and surface receptor expression of both class B (CD36) and A scavenger receptors (Figure 1D). Notably, these regulatory events led to sustained levels of decreased oxLDL internalization via macrophages. Additional studies confirmed that both the shell and the core components of the NPs are essential for the inhibition of oxLDL uptake. The results suggest that the NP shell played an integral role in the extracellular interaction between the NPs and the macrophage, whereas the core played a stronger intracellular role causing the down-regulation of scavenger receptors. The cumulative result of the interactions between the NP shell and core with extracellular and intracellular domains of the macrophages led to the overall inhibition of oxLDL uptake.

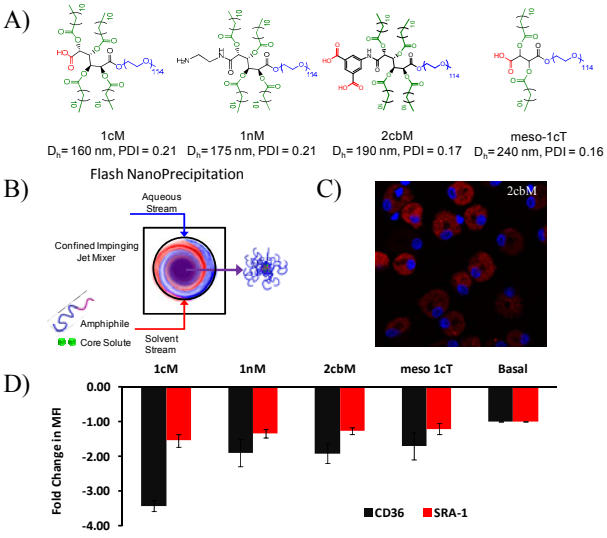

Figure 1. NPs (structures seen in part A) fabricated via the flash nanoprecipitation process (B) are taken up by macrophages (C) which leads to the down-regulation of surface scavenger receptors (D) and the inhibition of oxLDL uptake.

Conclusions: The AM NPs lower oxLDL uptake in macrophages due to a combined mechanism of action associated with both class A and B scavenger receptors. The initial NP interaction which occurs with class A scavenger receptors is likely dictated by the NP shell chemistry, which leads to internalization of the NPs. This then enables intracellular interactions with the NP core that facilitates the down regulation of surface and gene expression of both class A and B scavenger receptors. The overall effect of this cascade of scavenger receptor events leads to the prolonged inhibition of oxLDL uptake. Understanding the mechanism of action provides a promising strategy for the design and development of therapeutic drugs for the management of atherosclerosis.

References:

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