Nanoscale Assembled Polymers for Nuclear Targeted Biomolecular Delivery in Macrophages: Multifunctional Biomaterial Candidates for Management of Atherosclerosis

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Statement of Purpose: Excessive uptake of oxidized low density lipoprotein (hoxLDL) in macrophages leads to formation of foam cells, which triggers the escalation of atherosclerosis. This study investigates the dual functionality of nanoscale amphiphilic polymers (NAPs) to (a) block uptake of hoxLDL mediated by scavenger receptors; and (b) deliver Liver-X Receptor (LXR) agonist causing cholesterol efflux as high density lipoproteins (HDL). For the drug, we chose a specific synthetic liver-X-receptor agonist (s-LXRA), GW3965, which has been shown to upregulate reverse cholesterol transport through an increase in ATP Binding Cassette A1 (ABCA1), a cell membrane protein that shuttles excess cholesterol out of the cell as HDL. We have designed various configurations of the NAPs based on variations in the size, charge, and branched architectures of biocompatible components – poly(ethylene glycol) (PEG), mucic acid and aliphatic acid. Configurations that can basally inhibit hoxLDL uptake in human THP-1 macrophage cells include 1cM (presentation of one carboxylate from hydrophobic mucic acid chain of NAP).
Methods: THP-1, human monocytes, were differentiated into macrophages 72 hours before experimental use through exposure to phorbol 12-myristate 13-acetate (PMA). Macrophages were incubated with 10^{-8}M of s-LXRA (GW3965) and/or NAPs (10^{-6} M) for 24 hr at 37 °C to determine the extent of s-LXRA internalization. The internalized s-LXRA was visualized on a Leica TCS SP2 multiphoton microscope. Macrophages were also exposed to different test conditions of variable NAP chemistry and two forms of drug incorporation (drug encapsulated versus non-encapsulated), and then analyzed to determine gene expression with the use of the RNeasy Mini kit from Qiagen. DNA expansion was conducted with Quantitative RT-PCR on a Roche light cycler with a B-actin housekeeping gene. Effects of the NAPs on the uptake and efflux of fluorescently labeled hoxLDL (10ug/mL) were assessed in THP-1 macrophages.
Results: The cellular internalization of s-LXRA following NAP based delivery was visualized (Fig 1). Drug internalization through the 1cM NAP was significantly increased over controls (non-encapsulated drug). The bioactivity of internalized s-LXRA was confirmed in terms of the upregulation of two genes related to the LXR signaling cascade. Both genes, ABCA1 and LXR, were significantly upregulated only when the encapsulated drug s-LXRA was delivered via the 1cM NAPs (Fig 2). This formulation also led to significant reduction in the net hoxLDL accumulation within cells.

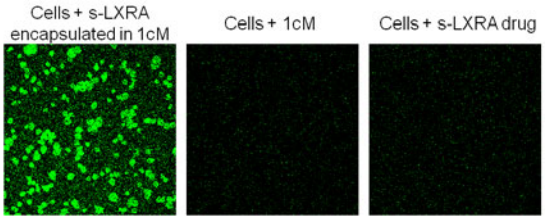

Figure 1: Two-photon fluorescence microscopy of cell-internalized s-LXRA drug within THP-1 macrophage cells. Only the drug encapsulated within the charged nanoscale amphiphilic polymers (1cM) enhanced intracellular delivery of the drug.

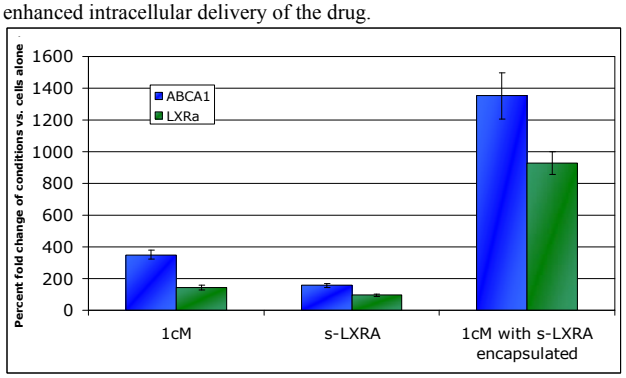

Figure 2: Expression of two key atherogenesis related genes, ABCA1 and LXRa, in THP-1 macrophages after exposure to non-encapsulated drug versus NAP-encapsulated drug.

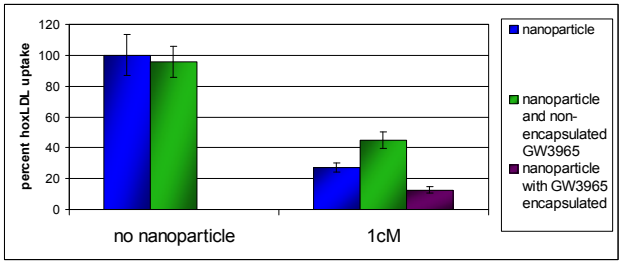

Figure 3: The effect of the NAP carriers and s-LXRA drug on total hoxLDL content (influx minus efflux) of THP-1 macrophage cells. The combination of NAPs and encapsulated form of drug nearly abolished any hoxLDL accumulation.

Conclusions: A nanoscale polymer-based encapsulation method was proposed to affect enhanced intracellular delivery of a drug agonist to a nuclear, liver-X-receptor in human macrophage cells *in vitro*. The polymers reduce the basal accumulation of hoxLDL by blocking scavenger receptors. Further, the polymers delivery maintains the bioactivity of the drug in terms of its gene expression and enhanced inhibition of cholesterol transport after a very short time period. The enhanced delivery may allow for use of high potency formulations of similar drugs for targeted delivery to macrophage cells in atherosclerotic lesions.