Multi-Functional Nanoparticle Development for Glycocalyx Heparan Sulfate Regeneration on Cultured Endothelial Cells

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Statement of Purpose: Cardiovascular diseases are the leading cause of death in the world, killing more people every year than all forms of cancer combined. The precursor to the majority of cardiovascular diseases is atherosclerosis, the accumulation of plaque initiated by the oxidation of low-density lipoproteins, ultimately leading to hypertension and stenosis. Current treatments include physically bypassing the plaque or using statin-type drugs to lower blood cholesterol levels, but are riddled with complications and side effects. Furthermore, these treat only symptomatic and diagnosed atherosclerosis and are not used for preventative measures.

There is evidence indicating that atherosclerosis coincides with degraded endothelial Glycocalyx (GCX). Therefore, GCX is a potential area of focus in prevention of atherosclerosis. The GCX is a porous, brush like structure composed of glycoproteins and glycosaminoglycans (GAGs) that line the inner blood vessel wall. It regulates blood vessel wall endothelial cell signaling and remodeling to protect vessel tone and acts as the barrier between the lumen and vessel wall (Ebong EE. Integr Biol. 2014;6:338-348). Here, we are focusing on the most abundant component of the GCX, heparan sulfate (HS), by delivering the GAG itself as well as the gene for the N-deacetylase/N-sulfotransferase-1(NDST-1) enzyme that is responsible for HS production. Gold nanoparticles offer a nanoscale carrier with biocompatibility as well as a wide array of functionalization potential, which can be tailored specifically for our application of GCX regeneration and gene delivery in areas where GCX is degraded and pro-atherosclerotic. In this paper we specifically show the (1) fabrication and (2) effects of HS-conjugated nanoparticles on healthy and damaged endothelial GCX. By restoring proper GCX structure, we hope to also restore its function and deter the onset of atherosclerosis and cardiovascular disease.

Methods: Rat fat pad endothelial cells were cultured for their robust GCX. Damaged GCX was modeled through either culturing the endothelial cells in media with no protein or in heparinase III, leading to either collapse or shedding of the GCX (Fels J. Cell Tissue Res. 2014;355:727-737). Cells with healthy or damaged GCX were fixed in paraformaldehyde and gluteraldehyde. On the fixed endothelial cells, bovine serum albumin and heparan sulfate were stained to visualize the GCX as a whole and the specific HS component, respectively. Stained GCX was quantitatively measured under confocal fluorescence microscopy. Gold nanoparticles were synthesized by reduction of $AuCl_4$ and stabilized with tetrakis(hydromethyl)phosphonium chloride, then PEGylated using dual functionalized thiol-PEG-R to which HS and fluorophores were conjugated (Kumar R. Transl Cancer Res. 2013;2:228-239). Particle characterization was done through electron microscopy, FTIR, DLS, zeta potential, and toxicity measured through MTS assays.

Results: Gold nanoparticles of 2-3 nm were synthesized and PEGylated, then conjugated with AF647 fluorophores. The particles exhibited stability and biocompatibility when incubated with RFPEC. As expected, the RFPEC expressed abundant GCX, as shown by BSA and HS staining. Both BSA and HS staining reveal that the GCX decreased in thickness and coverage after the protein removal and heparinase III treatments. The nanoparticles passively targeted these RFPEC with damaged GCX. This can be seen in Figure 1 where 1A is healthy cells expressing GCX stained with BSA antibody, 1B is low-serum-conditioned cells with BSA stain, and 1C is heparinase treated cells with HS stain. Adding HS to the media of GCX-degraded RFPEC regenerated the HS coverage in the sample. We are investigating the extent of GCX recovery in RFPEC treated with the NDST-1 gene and, eventually, nanoparticles conjugated with HS or designed to deliver the NDST-1 gene.

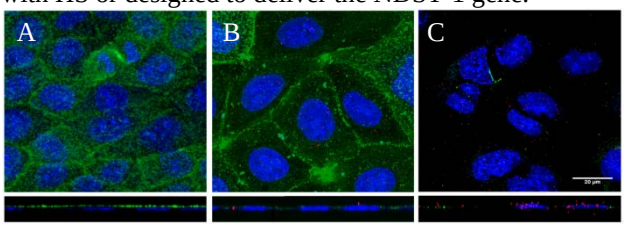

Figure 1: Gold nanoparticle uptake in healthy (A), collapsed (B), and degraded (C) GCX models. Blue is DAPI, green is BSA or HS, and red is nanoparticles. (A) and (B) are stained with BSA antibodies while (C) is an HS stain.

Conclusions: The nano-scale gold carriers function as a potential candidate for drug delivery and imaging for GCX regenerative applications. PEGylation decreased nonspecific cellular uptake and the degradation models provide a discontinuity in the GCX layer that allow the nanoparticles to passively target areas of interest. Delivery of the HS related therapeutics boosted recovery of the damaged GAGs and restored the barrier function of GCX. The modular aspect of the gold nanoparticles allow for easy translation to the other components of the GCX for a more comprehensive, generalized therapeutic mixture that can target all of the GAGs. The recovery of the GCX can prevent the initiation of atherosclerotic plaque development and lower cardiovascular disease related deaths.

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