Endothelial Glycocalyx Conditions affect Cellular Uptake of Gold Nanoparticles

Abstract:
Cardiovascular diseases are facilitated by endothelial cell (EC) dysfunction and coincide with EC glycocalyx (GCX) coat shedding. These diseases may be deterred by targeting medications to affected vascular regions using circulating nanoparticle drug carriers. The objective of the present study was to observe how EC-targeted delivery of 10nm polymer-coated gold nanoparticles (PEG-AuNP) are affected by the endothelial glycocalyx (GCX). Rat tail pad endothelial cells were chosen due to their robust glycocalyx, verified by fluorescent immunolabeling of adsorbed albumin and integrated heparan sulfate (HS) chains. Confocal fluorescent imaging revealed 3µm thick glycocalyx layer, covering 75% of the EC surface with abundant HS. This healthy glycocalyx deterred uptake of PEG-AuNP as expected, because glycocalyx pores are typically 7nm wide. Additional glycocalyx models tested included a collapsed glycocalyx by culturing cells in reduced protein media, a degraded glycocalyx by addition of heparinase III enzyme, and a recovered glycocalyx by supplementing exogenous HS into the media after degradation. Both collapsed and degraded glycocalyx led to decreased glycocalyx thickness of 2nm, but the collapsed model did not affect coverage or HS content. The heparinase III degradation caused the HS thickness to drop to 0.7um and HS coverage to decrease to 10% of the healthy glycocalyx. Both dysfunction models retained 6-7 fold more PEG-AuNP compared to the healthy control. The collapsed glycocalyx permitted nanoparticles to cross the glycocalyx into intracellular spaces, while degraded glycocalyx trapped the PEG-AuNP within the glycocalyx. The repaired glycocalyx model partially restored HS thickness to 1.2um and 44% coverage, in addition to reverse nanoparticle uptake back to baseline levels.

Methods:
Rat tail pad endothelial cells (RFPEC) were cultured and immunolabeled with bovine serum albumin and heparan sulfate antibodies under various conditions.

Table 1: Glycocalyx Treatments:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Low Serum</th>
<th>Hep III</th>
<th>Hep III + HS</th>
<th>Control + HS</th>
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<tr>
<td>HS (µg/mL)</td>
<td>550</td>
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<td>PEG-AuNP</td>
<td>59</td>
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Results: Glycocalyx characterization

Results: Gold nanoparticle uptake

Figure 2: Confocal microscopy images of cultured RFPEC to visualize the GCX. A) Control, BSA stain. B) Low serum, BSA stain. C) Control, heparan sulfate stain. D) Heparinase III treated, heparan sulfate stain. Treatments of low serum and heparinase III decreases GCX expression. Scale bar 20 µm for A-D.

Figure 3: Quantification of GCX modifications: Left: Overall GCX thickness and coverage from BSA stains. Low serum and Hep III treatment decrease GCX thickness (p<0.001) but had no effect on the coverage. Right: Heparan sulfate thickness and coverage. Heparinase III decreases HS coverage on the RFPECs (p<0.001), but addition of Heparan Sulfate (59 µg/mL) partially recovers it (p<0.001). Data = mean ± SEM, N=3

Conclusions:
- PEG-AuNP is biocompatible and the attachment of fluorophores allow for imaging and tracking of nanoparticles within the GCX
- Heparinase III and low serum treatments are suitable to model GCX damage, while addition of heparan sulfate can mimic regeneration
- GCX collapse and degradation result in different uptake patterns
- GCX dysfunction leads to AuNP uptake and healthy/regenerated GCX prevents foreign particle interaction

References:
Cohen, HS, Robbins Pathologic Basis of Disease 1999
Engel, EE, et al, Integrative Biology, 2014
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Figure 1: Synthesis, TEM, DLS of PEG-AuNP

Figure 4: Confocal microscopy images of PEG-AuNP (red) incubated in various GCX conditions. A) Control. B) Low serum. C) Heparinase III. D) HS treatment after HepIII degradation. E) Relative uptake of AuNP for various GCX conditions compared to healthy GCX. PEG-AuNP uptake is significantly increased (p<0.001) in both damaged GCX models but decreases to normal levels after addition of HS. Scale bar 20 µm for A-D.

Figure 5: AuNP distribution. Top: Cross sections of RFPEC incubated with AuNP under various GCX conditions. Bottom: Histogram of red fluorescence within treated RFPEC. Distance is the length from bottom of cell. Pale green: average GCX location.