Correlative Light and Electron Microscopy to Determine the Fate of Polyelectrolytes in Porcine Arteries

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Statement of Purpose: Revascularization procedures are often plagued with complications related to the reobstruction of the treated artery (restenosis) resulting from injuries induced during the procedure to the vascular wall. Our lab was the first to demonstrate that a nanoscale layer-by-layer (LBL) self-assembly of two polysaccharides, hyaluronan (HA), a polyanion, and chitosan (CH), a polycation permits the construction of thin films containing macromolecules, such as proteins, enzymes, or nucleic acids, with targeted properties onto a variety of substrates (i.e. stents).[1,2] The combination of the prodrug approach and the LbL technique, using a hyaluronan (HA) ester prodrug of paclitaxel as the polyanion to construct polyelectrolytes multilayers with chitosan (CH) have shown to restore blood compatibility of injured arteries, alleviating the risks of post-angioplasty restenosis of blood vessels and offer better control of drug delivery.[3,4] As well, by combining confocal and transmission electron microscopy, it allowed us to monitor the deposition and localization of polyectrolytes through intact and damage porcine arteries from the micron to the nanometer scale while maintaining spatial orientation.

Methods: Near infrared emitting (NIR) InGaP/ZnS quantum dots (QDs) will be used to guide us to monitor the fate of CH and HA on porcine arteries. QDs were either electrostatically or covalently linked to CH and HA (QD-CH and QD-HA) through the formation of an amide bond[5] as previously reported. The CH/QD-HA, CH/HA/QD/HA/CH, CH-QD/HA and CH/QD/CH/HA self-assemblies (5 bilayers) were deposited directly on damaged porcine aortic samples. Each QD or polyelectrolyte layer was alternatively deposited on the sample for 15 min. To avoid diffusion of the polymers during handling and storage of treated arteries, specimens were snap-frozen in 2-methylbutane using liquid nitrogen and kept at -80°C until used. Tissues were cut into 10 µm thick sections with a cryostat and mounted onto a glass slide prior to fixation with 4% paraformaldehyde. The sections were imaged using an Axiovert inverted microscope equipped with an LSM 510 confocal system (Zeiss). For electron microscope studies, tissue was fixed in 3 % glutaraldehyde in phosphate buffer @ pH 7.4 with sodium cacodylate. The samples were then diced and postfixed with 1 % OsO_4, dehydrated with acetone and embedded in EPON. Tissue were cut into 90-100 nm sections and stained with uranyl acetate and lead citrate. Images observed with FEI TECNAI 12.

Results:
After CH/QD/CH/HA self-assembly on porcine artery sections, laser confocal images (Fig 1 A) show that chitosan is able to diffuse through the inner wall (intima) of the artery all the way to the outer wall (adventitia). Z-sectional images were collected in order to reconstruct a 3D image of the treated tissue and this allowed us to measure the amount of CH-QD contained within the tissue (Fig 1 B). Visualization of polymer diffusion using electron microscope revealed also that chitosan can penetrate all the way to the media-adventitia section of the tissue (Fig 1 C) but more impotantly at higher magnification we were able to observe that there is some interactions with smooth muscle cells nuclei and CH-QDs (Fig 1 D). These preliminary results suggest the use of QDs can provide unique fundamental information about polyelectrolytes interaction with biological organelles.

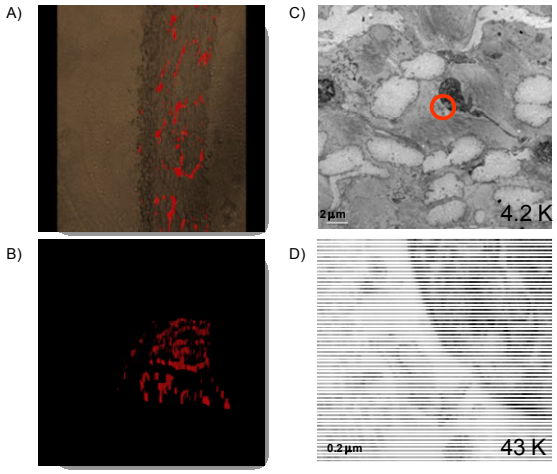

Figure 1: Images of porcine artery tissue treated with CH-QD-CH-HA self-assembly using (A-B) laser confocal and (B-C) electron microscope.

Conclusions:
By functionalizing NIR QDs to CH and HA polyelectrolytes, we were able to understand the internalization mechanistic pathway through porcine arteries using light and electron microscopy. These studies will hopefully guide researchers into developing better therapeutic drug delivery systems.

References:
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[4]Thierry, B. *et al. Journal of Biomedical Materials Research Part A* **2005**, *75A*, 556-566.
[5]Sandros *et al. Advanced Functional Materials* **2007**, *17*, 3724.