Assessing the Calcification Potential of Engineered Vascular Grafts with Surface Enhanced Raman Spectroscopy

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Statement of Purpose:
Tissue engineered vascular grafts (TEVGs) are a promising alternative to surgery for the treatment of thrombotic occlusion of small diameter (< 6 mm) arteries. However, graft calcification is a concern that is often overlooked. Graft stiffening due to calcification can contribute to graft stenosis and reduced long-term patency [1]. Thus, the goal of this study is to develop a system that can non-destructively detect both early- and late-stage calcification markers with vascular cells on fibrous scaffolds, using dispersive Raman and surface enhanced Raman spectroscopy (SERS), respectively. The results are verified using histological techniques. To the best of our understanding, this project will be the first use of the highly sensitive SERS technique to detect early markers of mineralization in electrospun TEVGs.

Methods: Electrospun poly(ε-caprolactone) (PCL) scaffolds were prepared with 1.54±0.11 µm diameter fibers. Cell-induced calcification was determined using smooth muscle cells (SMCs) isolated from rat abdominal aortae following IACUC approved procedures. SMCs were seeded at 7,500 cells/cm^2 on glass coverslips and electrospun sheets. Cells were cultured in regular DMEM with 10% serum. As a positive control, we incubated SMCs with calcification media (2.6 mM Ca^{2+} and 2.2 mM PO_4^{3-}). Markers of cell phenotype and early-markers of calcification (e.g., osteopontin and Runx2) were analyzed with PCR and immunofluorescence (IF).

We started with analysis of late-stage calcification using dispersive Raman and Von Kossa histological staining. For early-stage biomarkers of calcification, we have synthesized and characterized SERS-active nanoparticles. Gold nanoparticle seeds of 11.1±1.1 nm diameter were prepared by a citrate reduction method, and used as nucleation seeds to synthesize 60-65 nm diameter star-like gold nanostructures for enhancing SERS intensity. Antibody-conjugation is performed using carbodiimide chemistry.

Results: We have established a baseline with negligible SMC-induced calcification after up to the 21 days of culture (not shown). With calcification media, SMCs had less calponin staining, were less spread, and exhibited more mineralization than those grown in regular media (**Fig. 1 and 2**). Staining with osteopontin did not show a noticeable difference between conditions (not shown).

Dispersive Raman analysis demonstrated that 3-D maps can be created and show cells and PCL fibers (**Fig. 3**). Calcification can be detected using a calcification peak detected at a 960 cm^{-1} Raman shift. In preliminary data, we have generated silica coated Raman active particles, added PEG moieties, and demonstrated that the hydrodynamic radius increases slightly, 93.9 to 109.8 nm when RGD peptides are conjugated to the nanoparticles (**Fig 4**). The SERS work with conjugated antibodies for early-calcification markers is ongoing.

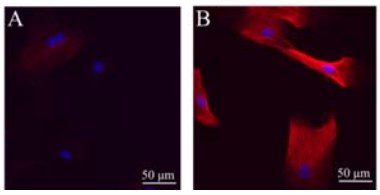

Fig. 1. Calponin-stained SMCs grown on coverslips with calcification media (A) and regular media (B).

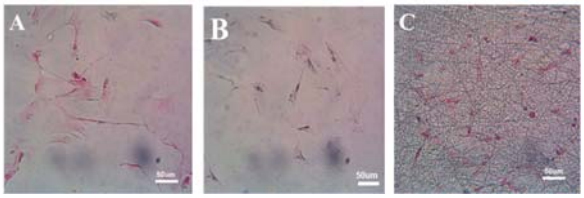

Fig. 2. Histology images of SMCs incubated on coverslips (A, B) or PCL meshes (C) stained with Von Kossa. (A, C) SMCs grown in regular media.

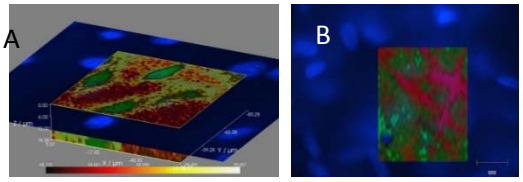

Fig. 3. Dispersive Raman map of SMCs grown on a MgFl coverslip (A) and PCL mesh incubated for 35 days (green is cellular material, red PCL fibers) (B).

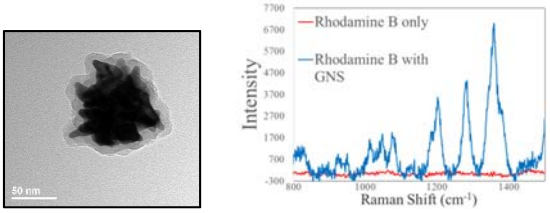

Fig. 4. (A) TEM image of silica-coated gold nanostars, and (B) Raman spectra for Raman active nanoparticles

Conclusions: This study demonstrates that Raman techniques can be adapted to analyze calcification in vascular conduits. In addition, SMCs grown in calcification media exhibit changes in contractility, spreading, and mineralization compared to those grown in regular media. In conclusion, this study represents the first steps toward non-destructive screening of TEVGs for calcification potential with SERS [2].

References:
[1] Mugnai, D., et. al., The Journal of thoracic and cardiovascular surgery 146, 400, (2013).
[2] Zavaleta, C.L., et. al., PNAS of the United States of America 110, E2288, (2013)