Lead-Based Nanoparticle Contrast Agent for Vascular MicroCT Imaging

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INTRODUCTION:
Vessel detection and monitoring is especially important for a variety of pathologies. Historically, vessels were assessed via histology which can be significantly influenced by sectioning bias. Microcomputed tomography (micro-CT) has revolutionized orthopaedic research by permitting non-destructive three-dimensional imaging to assess bone structure in pathological and treated milieus. Because it is a 3D modality it better captures tissue structure in totem than histology. However, it does not visualize soft tissues like vascular structures within organs unless contrast agents are used. The contrast agent's properties like viscosity, radiopaqueness, particle size, and hydrophilicity affect the quality of the images produced. An effective post-mortem vascular contrast agent must have a diameter small enough to enter capillaries, remain within the vasculature over an extended period of time, and be radiopaque enough for easy distinction from bone and other tissues via thresholding. The objective of this project is to determine the efficacy of a novel lead (II) carbonate nanoparticle (LNP)-alginate nanocomposite gel for use as a vascular contrast agent by in vitro characterization and mouse perfusion.

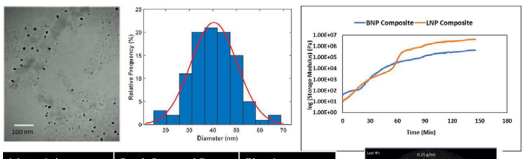
METHODS:
All animal procedures were approved by the applicable IACUC. The TEG-capped nanoparticles and nanocomposite gel were created and characterized in vitro to determine ideal nanoparticle concentration (n=3/group). Fist, the capped nanoparticles were analyzed with powder X-ray diffraction, dynamic light scattering, and transmission electron microscopy to determine particle size. The nanoparticles (0.1 to 0.4g/mL) were combined with aqueous Na-alginate polymer (ALG, 1% w/v) in the presence of d-(+)-gluconic acid δ-lactone (GDL) to form a gel that was mechanically tested via storage modulus and compressive testing in comparison to a previously developed barium sulfate nanoparticle composite (BNP) . GDL lowers the mixtures pH thus stripping the nanoparticles of their capping agents and making them available to interact with the ALG and form a stable gel. The radiodensity of the gels was also analyzed using micro-CT. Based on the resulting data, a pilot test was performed on 12 week-old C57BL6 mice (n=1-2/group). The mice were anesthetized and then underwent transcardiac perfusion (5mL/min) of heparinized PBS (15U/mL), 10% neutral buffered formalin, and contrast agent. After full perfusion, GDL was injected into the heart as well as the descending aorta and inferior vena cave to initiate gelation. Mice carcasses were left to gel at 4C over night. Then the tissues were harvested, placed in 10% neutral buffered formalin overnight at room temperature, and stored in 70% ethanol for 3 days until microCT scanning (10um resolution). Between each step, the body/tissues were imaged to determine stability. The microCT scans were assessed in Dragonfly for the ability to segment bone and vessels via threshold from a single scan. These samples are currently in processing for histological evaluation.

RESULTS SECTION: LNPs capped with TEG were on average 40 ± 10nm via TEM, which is well within the range to transverse capillaries (Figure 1). This size range was confirmed by PXRD and DLS (data not shown). LNP-ALG gelation times were similar to a prior formulation using BNPs and around 30 to 60minutes, which should be ample time to perfuse a full mouse or rat. Further, these gels were 2x as strong as the BNP-ALG formulation and 4x as strong as the current industry standard, microfil. Bulk, microCT scanning with a reference bone indicate that 0.4mg/mL should be adequately radiopaque for clear segmentation from bone. Pilot in vivo testing demonstrated that the gel is stable through all processing steps (i.e. dissection, post-fixation, and 70% ethanol storage) (Figure 2). MicroCT scanning confirmed the correlative segmentation with increasing LNP concentration (Figure 3). As demonstrated in bulk, 0.4g/mL LNPs were significantly more attenuative than bone. Thus segmentation by a simple threshold yielded fairly good separation of bone and vessels not only from soft tissues but from each other. However, it was not perfect. Very small vessels and the outer perimeter of most larger vessels exhibited partial volume and beam hardening effects causing these voxels to be binned in with bone.

DISCUSSION: LNPs show great promise to be an effective post-mortem vascular contrast agent. At higher concentrations in an ALG matrix, the solution is still able to freely flow through vessels at a relatively low viscosity. Only after the addition of GDL to lower the pH will the LNPs be able to interact with the ALG to cause gelation which will be ideal for reaching all regions of a tissue of interest. Moreover the resulting gel is exceptionally stronger than the current standard, microfil. However, at a 10um resolution there are many partial volume effects that limit its applicability for small vessel imaging. Work is on-going to better validate infilling and segmentation with higher resolution scans and histology as well as optimizing perfusion/gelation protocols.

SIGNIFICANCE/CLINICAL RELEVANCE: Being able to image vessels post-mortem with a 3D modality like microCT would greatly enhance research efforts and improve result accuracy. While there are some existing contrast agents, most are not ideal for reasons like high viscosity, inability to transverse capilarries, and inadequate contrast from bone. LNPs in ALG represent a promising solution.

IMAGES AND TABLES:



Material	Peak Stress, kPa	Elastic Modulus, kPa
LNP–alginate gel	55 ± 5	318 ± 4
BNP-alginate gel	25 ± 1	112 ± 2
12% gelatin	91 ± 5	604 ± 5
Microfil	12 ± 1	60 ± 1

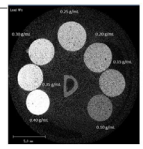


Figure 1. Characterization of LNPs. LNPs were adequately small to potentially transverse capillaries. They exhibit increasing gelation for 60 to 90 minutes and are 2x stronger than the same composite made with BNPs and 4x stronger than microfil. The composite's radiopacity increases linearly with LNP concentration. A concentration of 0.4g/mL or above should be adequate for segmentation from bone.

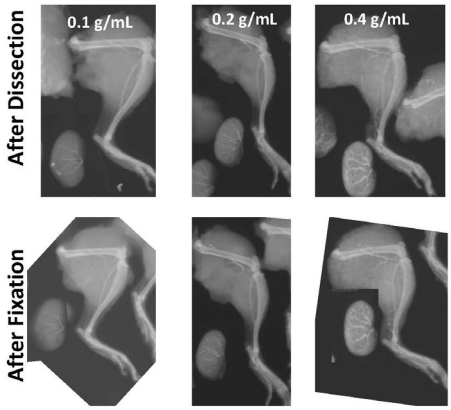


Figure 2. Demonstration of Initial Radiopacity & Retention. Xrays at all steps showed essentially identical infilling.

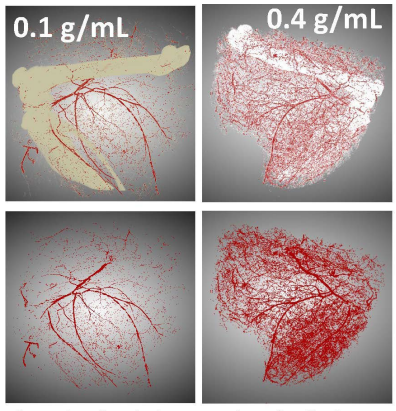


Figure 3. MicroCT Segmentaion of a Single Scan by Threshold. While not perfect, there was ample contrast in most of the vascular network to separate bone and vessels.