X-ray Phase Contrast Imaging of Soft Biomaterials in a Whole-Animal Model

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Statement of Purpose: Imaging soft biomaterials *in vivo* is a challenge as most conventional techniques fall short in resolution, penetration depth, or acquisition time. Also, many techniques require additional contrast agents to visualize biomaterials, which have risks associated with their use. Having the capability to observe whole biomaterial constructs while they are still implanted without introducing exogenous contrast would improve the process of developing them for clinical use. Monitoring material properties such as degradation, stability, interaction with surrounding tissue, and point of failure in real time would allow for more relevant information gathering and remove potential sources of error due to processing the tissue after sacrifice. Conventional x-ray imaging allows fast, high-resolution imaging at high penetration depth. However, the low absorption of soft biomaterials and surrounding tissue limits contrast in x-ray-based imaging methods. Previous studies have shown that techniques based on x-ray phase contrast (XPC) may enable imaging soft biomaterials in-vivo without contrast agents. Previous studies employing XPC used a synchrotron source for high-energy x-rays, resulting in useful images, but this is not practical for widespread use[1]. The purpose of this paper is to evaluate the capability of imaging soft biomaterials through XPC tomography in a small animal model using a commercially available, in-lab liquid metal jet source.

Methods: Alginate microbeads were used as a model a soft biomaterial. The microbeads were implanted into a rat omentum model in the presence of LPS which us used to stimulate an inflammatory response. Briefly, ultrapure alginate from Polysciences (Warminster, PA) was extruded in droplet form into a $CaCl_2$ solution to form ionically crosslinked beads. In addition to ionic crosslinked beads dual (ionic and covalent) crosslinked beads were formed by adding a 2-aminoethyl acrylate (AEMA) group through NHS/EDC coupling to the alginate, then extruding it in droplet form into a $CaCl_2$ solution with a photoinitiator, and crosslinking upon exposure to UV (365 nm)[2]. An additional set of multilayer alginate beads formed using LVM ultrapure alginate from Nova-Matrix (Oslo, Norway)[3]. The beads were then washed with a $CaCl_2$/saline solution and added to a .1 w/v poly-L-ornithine (PLO) solution, followed by a wash with more LVM ultrapure alginate, and added to a second crosslinking solution. This generated beads of both ionic and dual crosslinking with a thin PLO layer as well as an outer alginate layer. 100 beads were implanted into the omenta of Lewis rats. Animals were euthanized at either 1 or 3 weeks. Each animal was imaged using an XPC imaging system. Cone-beam tomography was performed using a MetalJet D2 Excillum X-ray source operated at 70 kV 100W with ~10 um anode focal spot. Emission spectra from the liquid-metal ExAlloy anode is formed mostly by the characteristics Kalpha lines (Ga -9.25keV, In-24.2 keV and 25.3 keV) superimposed on the 70 kVp bremsstrahlung. Projection images (total 380 projections with 0.5 deg step) were recorded using 2304x2940 16-bit Shad-o-Box Teledyne Dalsa camera. Camera pixel size is 49.5x49.5 um. CT geometry used in our experiments (source-object distance is 84 cm; object-detector distance 40 cm) provides 33.5 um object pixel size (actual resolution ~70 um). The images were reconstructed and rendered in 3D using Leica LASX software. After imaging, the omenta were explanted, fixed, paraffin embedded, and sectioned. H&E staining was performed on the tissue sections. Stained sections were imaged using a Leica DMI6000 microscope, and images were registered to the XPC tomography slices.

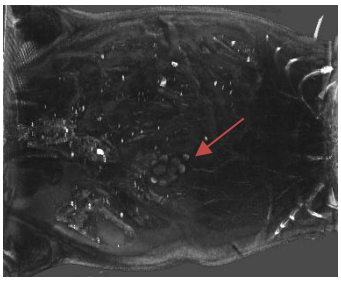

Figure 1. XPC image of implanted microbeads

Results: XPC imaging of the implanted beads shows that soft biomaterials can be clearly imaged without exogenous contrast agents. Single layer beads which were only ionically crosslinked did not survive the inflammatory response. Beads from the other three groups did survive, which was reflected in histological analysis. At the time of explantation, the beads were intact, but histological processing caused the sliced beads to collapse, leaving circular voids where the alginate was previously located. Inflammatory response was confirmed by histology, but not visible in the XPC reconstruction. Comparing the tissue sections and the XPC reconstructions shows that single layered alginate beads which survived the inflammatory response are visible as rounded protrusions from the native tissue but cannot be fully distinguished. Beads with a layer of poly-L-ornithine were clearly visible in the XPC reconstruction and were able to be morphologically matched to the tissue sections in some cases.

Conclusion: Depending on biomaterial composition, 3-dimensional structure can be resolved within the tissue via XPC. Though not attempted in this study, this process should be applicable to monitoring biomaterials in-vivo over time and would provide significant amounts of additional data if utilized in biomaterials research.

References:

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