X-ray Phase Contrast Imaging of Hydrogels for Tissue Engineering

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Statement of Purpose: Hydrogels have shown promise for many tissue engineering applications; including as a method for cell encapsulation. It is difficult to image hydrogel scaffolds as they generate little contrast with conventional technologies and are difficult to process with histological methods. Recently, techniques based on X-ray phase contrast (PC) have been investigated for biomedical engineering applications because they can provide information about the X-ray refractive properties of samples which allows for hydrogel and soft tissue imaging without exogenous contrast agents.[1]The objective of this research is to evaluate mechanisms of X-ray contrast produced by hydrogel scaffolds and determine the extent to which X-ray signatures can be used to identify these scaffolds *in vitro* and in tissue. Both a synchrotron and benchtop X-ray phase contrast technique will be evaluated to assess the translation of the findings to a more clinically relevant system.

Methods: Alginate beads were synthesized using standard methods and either cultured in a perfusion bioreactor system[2] or implanted in a rodent omentum pouch model. Bioreactor beads were imaged *in vitro* in CT mode at multiple time points with both synchrotron and benchtop X-ray PC imaging systems. Explanted rat omental samples containing beads were imaged in water with the synchrotron X-ray PC imaging system.

The benchtop X-ray system utilized an in-line imaging scheme where the detector is placed several cm behind the sample in order to capture a single image with edge enhancement at material interfaces. For CT scans, 400 projection views were collected over 200^{o} angular range. The synchrotron system, Multiple Image Radiography (MIR),[3] CT data were acquired at 500 tomographic views over 180^{o} at X-ray energy of 20-keV at 11 angular analyzer positions over 8 μradians. MIR produces three separate images that depict the projected X-ray absorption, refraction, and ultra-small-angle scatter (USAXS) properties of the samples.

Results: Alginate beads cultured in the TPS bioreactor could be identified in the benchtop X-ray PC image. The beads generated contrast in MIR X-ray refraction images but not absorption (Figure 1 A-C). At later time points the beads displayed higher contrast due to mineralization deposited and were observed in both the absorption and refraction images (Figure 2A-C). Alginate beads were implanted in a rat omentum model. Beads could be identified in all three MIR images (Figure 3). Surrounding adipose tissue could be separated from the inflammatory response to the implanted beads based on absorption contrast (Figure 3A). In addition, the local encapsulation response could also be identified in the X-ray refraction image (Figure 3D).

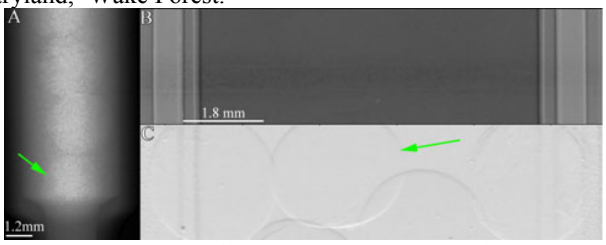

Figure 1: X-ray PC allows imaging of hydrogels. A) In-line benchtop X-ray PC image of alginate beads from a bioreactor B) MIR absorption and C) MIR refraction image of beads from synchrotron. Green arrows indicate bead edge.

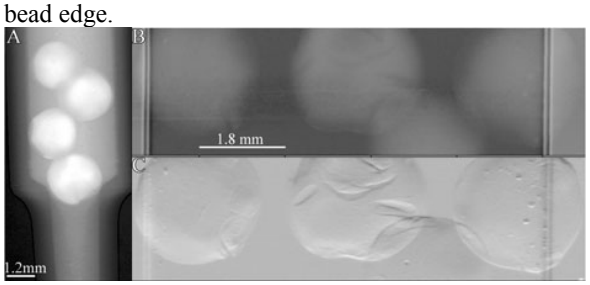

Figure 2: X-ray PC allows imaging of hydrogels and monitoring of mineralization. A) In-line benchtop X-ray PC image of alginate beads after 28 days in bioreactor B) MIR absorption and C) MIR refraction image of beads.

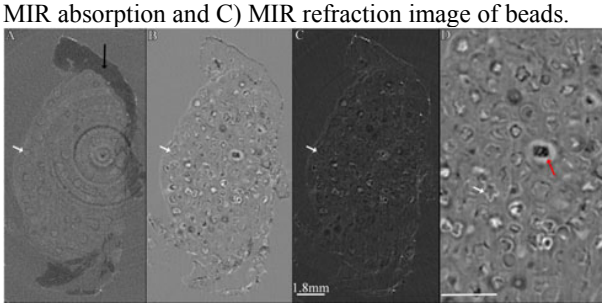

Figure 3: X-ray A) absorption, B) refraction, and C) USAXS slice from CT images of omentum containing alginate beads. D)Magnified region from B. White arrows indicates bead, black arrow indicates adipose tissue, and red arrow indicates local encapsulation response.

Conclusions: Hydrogel samples are invisible in traditional absorption based X-ray images due to their high water content. X-ray PC techniques enable imaging of these hydrogels based on differences in X-ray refractive index in both cell culture conditions and embedded in tissues. We were able to image hydrogels using both a synchrotron and benchtop system. These results provide a glimpse into the significant potential use of PC X-ray techniques to visualize and characterize hydrogels and engineered tissues *in vitro* and *ex vivo.*

References:

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[3] (Brankov JG. Med. Phys. 2006:33:278-289.)