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Low-dose phase-based X-ray imaging techniques for *in situ* soft tissue engineering assessments

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ABSTRACT

In tissue engineering, non-invasive imaging of biomaterial scaffolds and tissues in living systems is essential to longitudinal animal studies for assessments without interrupting the repair process. Conventional X-ray imaging is inadequate for use in soft tissue engineering due to the limited absorption difference between the soft tissue and biomaterial scaffolds. X-ray phase-based imaging techniques that derive contrast from refraction or phase effects rather than absorption can provide the necessary contrast to see low-density biomaterial scaffolds and tissues in large living systems. This paper explores and compares three synchrotron phase-based X-ray imaging techniques—computed tomography (CT)-diffraction enhanced imaging (DEI), -analyzer based imaging (ABI), and -phase contrast imaging (PCI)—for visualization and characterization of low-density biomaterial scaffolds and tissues *in situ* for non-invasive soft tissue engineering assessments. Intact pig joints implanted with polycaprolactone scaffolds were used as the model to assess and compare the imaging techniques in terms of different qualitative and quantitative criteria. For long-term *in vivo* live animal imaging, different strategies for reducing the imaging radiation dose and scan time—reduced number of CT projections, region of interest, and low resolution imaging—were examined with the presented phase-based imaging techniques. The results demonstrated promising capabilities of the phase-based techniques for visualization of biomaterial scaffolds and soft tissues *in situ*. The low-dose imaging strategies were illustrated effective for reducing the radiation dose to levels appropriate for live animal imaging. The comparison among the imaging techniques suggested that CT-DEI has the highest efficiency in retaining image contrast at considerably low radiation doses.

1. Introduction

As tissue engineering aimed at repairing damaged tissues and organs continue to advance, more sophisticated assessment and monitoring techniques are required to evaluate and/or optimize the success of outcomes. The information on the functionality, tissue regeneration, interaction, and integration of engineered constructs with the host tissue post-implantation is essential to the *in situ* evaluation of various therapeutic tissue engineering strategies. Such evaluation or assessment is critical for the strategies seeking approval for clinical application to patients; the approval process typically involves extensive and comprehensive longitudinal *in vivo* studies on large animal models and clinical trials on patients. Notably, conventional biochemical evaluation techniques (e.g., histochemical analysis), which have been widely used for various *in vitro* and *in vivo* studies, are invasive and destructive due to the need to sacrifice animals during the repair process for *ex vivo* analysis. As such, they are inadequate for use in clinically relevant and long-term *in vivo* studies. Furthermore, conventional *ex vivo* techniques cannot provide *de facto* and dynamic information about

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the *in vivo* repair process, which is the key to developing better regeneration strategies. Most importantly, such invasive assessment techniques have to be relinquished with the advance from animal to human studies. Therefore, the development of non-invasive assessment techniques with applicability to longitudinal live animal studies and eventually to human trials is urgently needed.

Biomedical imaging techniques such as magnetic resonance imaging (MRI) and X-ray imaging are forerunner candidates for non-invasive tissue engineering assessments. MR-based techniques have been explored for non-invasive monitoring in soft (i.e., cartilage) tissue engineering applications [1], but are currently limited to small animal models [1] or require exogenous contrast agents for applications to large animals [2]. Furthermore, MR-based techniques mainly focus on the evaluation of tissue growth [1] and have limitations with respect to *in situ* imaging and evaluation of polymeric tissue scaffolds [3]. With the discovery of synchrotron X-ray source that has superior brilliance, coherence, and a monochromatic (single energy) beam, robust phase-based X-ray imaging techniques have emerged [4–7] and led to breakthroughs in biomedical X-ray imaging of soft tissues and low density microstructures [8–14]. Phase-based X-ray imaging techniques rely on refraction-based and phase shift contrast mechanisms instead of absorption-based mechanisms that do not facilitate imaging of low density materials. In addition, synchrotron phase-based X-ray imaging techniques have the advantages of being free of contrast agents, providing high imaging resolution (down to 1–2 μm), using a high penetrating beam, and featuring highly sensitive imaging mechanisms. As such, synchrotron phase-based X-ray imaging offers great potential for non-invasive, longitudinal monitoring and assessment applications in large animal and human soft tissue engineering. Although the potential of X-ray phase-based imaging techniques and their capabilities for soft tissue engineering have been shown [3,15,16], these techniques are relatively new to the field and several issues remain to be addressed, particularly for use in longitudinal *in vivo* large animal and human studies.

Synchrotron diffraction-enhanced imaging (DEI), analyzer-based imaging (ABI), and propagation-based inline phase contrast imaging (PCI) techniques have been investigated for soft tissue imaging [17–19], particularly for cartilage and joint tissues [12,20–26]. Recently, these imaging techniques have been drawing considerable attention in soft tissue engineering applications [3,16,21,27–30]. Other phase-based X-ray imaging techniques such as edge-illumination and grating based imaging have also proven great potential for soft tissues [31–38] and low-density materials imaging [39–42]. It is noted that relatively high resolution imaging of large biological samples requires high X-ray energies and big field of view. This can makes the application of grating-based interferometry and edge-illumination techniques more challenging than DEI, ABI, and propagation-based PCI for non-invasive large animal tissue engineering assessments. As such, DEI, ABI, and inline PCI are currently more applicable than other phase-based imaging techniques to large animal imaging applications. Application of the DEI technique to cartilage tissue engineering assessment [3] suggested the necessity of computed tomography (CT) modality for successful and informative *in situ* imaging of engineered tissue scaffolds. Compared to non-CT modalities, however, CT requires collection of multiple 2D images; this often results in a longer scan time and a higher ionizing radiation dose that could prohibit widespread use of newly developed X-ray imaging techniques for live animal and human studies. Although some recent studies have tried to address these issues [31,43–45], the performance of various imaging techniques for clinically relevant *in vivo* studies involving imaging of large biological objects at safe doses, as compared in terms of imaging quality, radiation dose, and scan time, has not been explored and documented in the literature. Such comparison study provides a platform of different non-invasive assessment techniques for soft tissue engineering, which will greatly facilitate more realistic longitudinal and comprehensive *in vivo* studies with clinically translatable results that will advance tissue engineering technologies towards clinical patient trials and therapies.

In this study, synchrotron CT-DEI, CT-ABI, and inline CT-PCI (with different propagation distances) were investigated with respect to their potential as non-invasive X-ray monitoring techniques for soft tissue engineering applications. By exemplifying a cartilage tissue engineering application, the capabilities of these phase-based imaging techniques and their practical aspects for longitudinal *in vivo* large animal studies were examined and compared. This included evaluation and comparison of the techniques in terms of imaging features of importance to tissue engineering assessments, scan time, and level of radiation dose delivered. To reduce the scan time and radiation dose, three strategies of reduced number of CT projections, region of interest, and low resolution imaging were investigated and compared with the three imaging techniques. Imaging quality and quantitative contrast of visualized structural features were further probed to identify the most suitable and efficient imaging technique for application to longitudinal *in vivo* animal studies with the potential for possible extension to human trials.

2. Materials and methods

2.1. Sample preparation

The samples used in this study were prepared by following the procedure developed in our previous study [3]. Briefly, scaffolds were designed with a porous structure featured by a strand spacing of 1 mm and a perpendicular (0°/90°) pattern of strands in successive layers and fabricated from polycaprolactone polymer (Mw 48,000–90,000; Aldrich, St. Louis, MO, USA) on a 3D Bioplotter (Envisiontec, Germany). Polycaprolactone was melted at 75 °C in a cartridge of the Bioplotter and then dispensed through a 22G (400 μm ID) needle under a pneumatic pressure of 0.75 MPa on the Bioplotter stage layer by layer to form a three dimensional (3D) scaffold. The fabricated scaffolds were implanted into the lateral femoral condyles of stifle joints dissected from four-week-old cadaver piglets (prepared at the Western College of Veterinary Medicine (WCVM) and the Vaccine and Infectious Disease Organization (VIDO), Saskatoon, Canada, under the animal use protocol # 20110071 approved by the University of Saskatchewan Animal Research Ethics Board). In each joint, an osteochondral defect (4.5 × 4.5 × 2–2.5 mm depth) was created and filled with the polycaprolactone scaffold and covered with a piece of concurrently harvested periosteum tissue to fix the scaffold in place; this procedure was similar to the one commonly used in the *in vivo* implantation of cartilage tissue constructs in animals [46]. The implanted intact joints with all surrounding tissues (including skin) were submerged in Dulbecco's phosphate buffered saline (DPBS) and placed under vacuum for 10–15 min to eliminate any air that might have entered the joint cavity during the surgery. It is noted that any trapped air in the joint may cause artifacts in the images and does not represent the actual status of the joint in a living system.

2.2. DEI, ABI, and extended-distance PCI

DEI, ABI, and extended-distance PCI were performed at the Biomedical Imaging and Therapy-bend magnet (BMIT-BM) beamline [47], Canadian Light Source (CLS), Canada. The experimental optical setup followed the protocol reported in our previous study