Rapid Throughput, Seamless Imaging of Human Hip Joint Tissue Across Length Scales to Elucidate Emergent Structure-Function Relationships

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Introduction: There is great imperative to understand the etiology and progression of osteoarthritis, a multifactorial disease involving complex interactions between tissues. Joint tissues such as cartilage and bone comprise stimuli-responsive, or so-called smart, composite materials. Our working hypothesis is that, as a smart, composite material, human hip joint tissue exhibits unique, emergent structure-function properties indicative of health status. A current barrier to understanding emergent properties of tissues is the paucity of rapid throughput imaging technologies that allow for seamless bridging of structure-function relationships across length scales (10-2 - 10-9 m). This area of unmet need, identified by both the National Science Foundation as well as the National Institutes of Health [1,2], provided the impetus for this collaboration between industry and academia in which we aim to develop for life sciences applications leading edge imaging capabilities originally developed for high-throughput quality control in semiconductor industry.

Multibeam scanning electron microscopy (mSEM) uses a single electron optical column for multiple electron beam sources, allowing for scale up of imaging speed through both optimization of the multibeam source as well as detector design. The mSEM technology has the potential to increase image acquisition rates by orders of magnitude, allowing for the first time to our knowledge, rapid throughput imaging of anatomical tissue blocks, such as whole joints, with electron microscopic resolution. As a first step toward elucidating structure-function relationships in the composite tissue comprising the articular surface, subchondral bone and cortical bone of the femoral neck, we imaged human hip joint tissue resected during the routine course of hip replacement surgery (n=3, Cleveland Clinic IRB protocol 12-335).

Methods: Specimen Acquisition and Preparation: Femoral necks and heads, normally discarded after hip replacement surgery, were collected after sectioning into 1 mm thick sections by the Cleveland Clinic Pathology Department per IRB protocol guidelines. Tissues were fixed, undecalcified, in 2.5% glutaraldehyde, 4% formaldehyde in 0.2 M cacodylate buffer at 4° C. They were then processed for bulk embedding in Epon (Sigma Aldrich) or Poly(methyl methacrylate) (PMMA) ± OsO4 contrast agent. After iterative automated polishing to 600 grit with copious deionised water irrigation (Buehler), specimens were prepared for imaging. Between imaging steps, PMMA embedded specimens were selectively etched with 0.02 M HCl for 90 s and/or 10% NaOCl for 11 min per our previous protocols [3,4] to selectively image the inorganic or organic phase of the extracellular matrices from respective tissues of the joint complex.
Imaging: After imaging in reflection and for autofluorescence using laser scanning confocal microscopy, specimens were carbon coated and imaged with the prototype mSEM system, before and after etching steps.

**Results:** Using the mSEM prototype, in concert with laser confocal scanning microscopy, we successfully imaged joint tissue blocks containing complex tissue composites across length scales (10⁻² - 10⁻⁹ m) and in a rapid throughput manner (Figure). Selective etching of the sample reveals further details of intrinsic organic and inorganic tissue structure.

**Discussion:** Multibeam scanning electron microscopy allows for unprecedented, seamless zooming in on tissue and organ scale specimens (up to 10 cm in diameter) to decipher cellular underpinnings of tissue health and disease. Using novel sample preparation methods and correlative microscopy, this new imaging platform provides a means to bridge basic science approaches to understanding cell biology and clinical approaches to elucidate disease etiology. This is of particular relevance for understanding interactions in complex tissues such as human joints in space and time.

**Significance:** This study demonstrates the feasibility of seamless multiscale imaging from subcellular to organ (whole joint) length scales using a technology, originally developed for semiconductor industry, applied to the orthopaedics arena. This new platform for elucidating structure seamlessly across length scales and in cohort with other imaging modes lends itself for elucidation of emergent structure-function relationships key to joint physiology in health, disease, and as a function of natural aging processes.

**Figure.** Seamless multi-scale imaging of hip joint tissue, prior to etching step. (A) Transmitted light image of OsO₄ stained femoral head section, with greatest penetration of contrast agent (darkest) in cartilage (white dotted square) and marrow spaces of the subchondral bone (black dotted square). (B) Laser scanning confocal image of specimen shows autofluorescence (light areas) of subchondral bone and marrow spaces (ellipsoids) as well as cartilage (dark), with two visible cartilage defects (bright cracks, arrows). Scale bar 120 μm. (C) Large scale, tiled mSEM® image of same area with single field of view showing (sub-)cellular resolution of bone (D,E) and blood (F) cells without additional etching as well as an osteocyte (G) with additional etching.