Fluid Localization within the Rabbit Knee Varies with Synovial Fluid Volume and Flexion Angle

Introduction: Synovial fluid (SF) in diarthrodial joints exhibits a number of mechanobiological features. SF modulates joint biology and biomechanics by providing cytokines, enzymes, and lubricants. These biomechanical and biological factors regulate synoviocyte and cartilage biology, in turn altering the composition of SF. The biomechanics of SF in situ in joints are largely unexplored. During joint flexion, the joint capsule tissue deforms and the bulk of the SF may shift within the joint cavity. SF volume is increased variably with therapeutic injections, in diseases, such as osteoarthritis (OA), and after traumatic injury. Overall SF volume is an important physiological quantity, as the biological and biomechanical effects of SF molecules are concentration-dependent, and joint effusions may alter such concentrations. Micro-computed tomography (μCT) can provide high resolution 3D structural information but has not previously been applied to quantify and localize SF volume. This study tested the hypothesis that fluid infusion into the rabbit knee joint, as well as flexion, alters the joint space SF distribution.

Methods: Tissue Harvest and Storage. A single hind limb (3 right, 3 left) from individual (n=6) adult (~4.5kg) New Zealand white rabbits were used. Knees were obtained immediately after euthanasia and stored at ~80°C until use. Experimental Design. Rabbit knees were injected with volumes of (1) 0.05mL; (2) 0.25mL; and (3) 0.95mL of μCT at a flexion angle of 60°, and also subsequently imaged (with 0.95mL) at (4) 30° and (5) 90° flexion. Contrast Agent. Phosphate-buffered saline with 60% barium sulfate was injected, analogous to that used for a variety of in vivo contrast-enhanced radiologic studies. The intra-articular injection was administered into the joint space via a 22ga. hypodermic needle through the patellar ligament. After injection, the joint was gently flexed 10x to distribute the contrast agent throughout the joint space. μCT Imaging and Analysis. Knees were scanned in a Skyscan 1076 μCT scanner at (35μm)³ voxels. The reconstructed images were thresholded above 80 grayscale and the joint space was divided into quadrants anterior (A) vs posterior (P), and medial (M) vs lateral (L) along the patellafemoral groove for each scan (Fig. 1B). CTAn (Skyscan) was used to calculate the volume of contrast agent in each quadrant. Volumes were also normalized to the total volume in each knee to determine relative volume (%). Statistics. Data are expressed as mean±SEM. Measured volume was compared to that injected and over flexion angles by linear regression. Repeated measures ANOVAs were used to assess the effects of infusion volume and flexion angle on quadrant volumes (AL, AM, PL, PM) with Bonferroni-corrected post-hoc tests. 2-way ANOVAs were used to assess effect of side (M vs L) with volume and flexion. Results: The total fluid volume detected was a high proportion (85%) of the total injected (Fig. 2A). Fluid volume was maintained for scans at the different flexion angles (Fig. 2B).

Volume. With successive injections, the fluid volume in each quadrant at 120° flexion increased qualitatively (Fig. 1) and quantitatively (Fig. 3A). Fluid volume was localized primarily in AL and AM quadrants (Fig. 3AB). With increasing volume, the quadrant percentages shifted away from AM and towards PM (Fig. 3B).

Flexion. With increasing flexion, the fluid volume shifted posteriorly as seen qualitatively in 3D volume renderings (Fig. 4) and quantitatively (Fig. 3). At 30° and 60° flexion, fluid volume was localized primarily in the AL, as well as the AM, quadrants (Fig. 3AB). With flexion to 90°, fluid shifted posteriorly, to the PL and PM quadrants (Fig. 3AB).

Discussion: The shift in SF volume with infusion and flexion (Figs. 1,3,4) is a direct indicator of the mechanobiological fluid environment within the knee joint. Such changes in fluid volume likely stretch the joint capsule, which in turn may alter synovial cell biology as well as fluid and macromolecular transport across the synovium. The approach of assessing fluid volume with radiopaque contrast and μCT may be useful for a variety of experimental studies.

References: Smith+ Int J Rheum Dis ’87; Nugent+ Arthritis Rheum ’06; Levick Handbook of Physiol: Microcirc. ’84; Pelletier+ Arthritis Rheum ’01; Schmidt+ Arthritis Rheum ’07; Garrett+ Radiology, ’84.

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Fig. 1: Typical μCT slices (A) of ex vivo rabbit knees (right knee shown) with 0.05 (B.C), 0.25 (D.E), or 0.95mL (F.G) injected fluid volume in transverse (B,D,F) or longitudinal (C,E,G) sections at 60° flexion.

Fig. 2: (A) Measured vs injected vol. (B) Vol. vs flexion angle (n=18).

Fig. 3: Measured volume (A) and % volume (B) by compartment for 3 injection volumes (0.05, 0.25, and 0.95mL) and 3 flexion angles (60°, 30°, 90°: A: anterior, P: posterior, M: medial, L: lateral. * P<0.05. #,*, ♦, ♠: P<0.001 effect for side (#), injection volume (♦), side×injection volume (♠), flexion (♣), and side×flexion (♦).

Fig. 4: Typical volume rendering of fluid and bone at 3 joint flexion angles (A) 30°, (B) 60°, (C) 90° after 0.95mL fluid injection.