Assessing Inflammation in the Pathology of Knee Osteoarthritis

Zachary J. Koudys, Western University

Supervisor: Teeter, Matthew G., The University of Western Ontario
Co-Supervisor: Thiessen, Jonathan D., The University of Western Ontario
A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Medical Biophysics
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Abstract

Osteoarthritis (OA) is a joint disease that causes pain, stiffness, and loss of function. Inflammation of the synovium plays a role in the pathology of OA. Macrophages are the dominant immune cells in synovial tissue. Activated macrophages over-express the translocator protein (TSPO). \(^{18}F\)FEPPA is a 2\(^{nd}\) generation positron emission tomography (PET) tracer that can target TSPO with high specificity. Hybrid \(^{18}F\)FEPPA PET/MRI may enable accurate quantification of macrophage activity in vivo. In this work, \(^{18}F\)FEPPA tracer uptake in knee synovial tissue was measured ex vivo using autoradiography and was validated to correlate to the true macrophage activity determined by immunofluorescence (IF). A clinical \(^{18}F\)FEPPA PET/MRI protocol showed similar ability to quantify macrophage activation in vivo. This study suggests that \(^{18}F\)FEPPA PET/MRI may be an effective tool to investigate the role of inflammation in the pathology of OA or to validate the efficacy of future anti-inflammatory OA treatments.

Keywords

Osteoarthritis, Orthopaedic Imaging, Synovitis, Activated Macrophages, TSPO PET, Autoradiography, Immunofluorescence, \(^{18}F\)FEPPA PET/MRI, Inflammation,
Summary for Lay Audience

Osteoarthritis (OA) is the most common joint disease affecting nearly 4 million Canadians. Knee OA causes symptoms of pain and stiffness, leading to a decrease in mobility and quality of life. Pain in OA is usually caused by thinning of a smooth surface that lines the bones in the knee called cartilage. When the cartilage becomes too thin, knee bones begin to rub together and cause pain. Recent studies have shown a link between the body’s inflammation response and the loss of cartilage. We need a better understanding of how inflammation relates to the start of OA to be able to develop effective treatments. A new agent that can target inflammation called $^{18}$FFEPPA has been developed and may be useful to learn more about how inflammation affects OA. The goal of this work was to verify that $^{18}$FFEPPA could be used accurately to measure inflammation in the knee, without the need to take a sample of diseased tissue.

An experiment was designed to scan the inflammation in knees of patients with OA who were scheduled to have knee replacement surgery. The results from the scan were compared to measurements of inflammation taken directly from tissue removed during the patient’s knee replacement surgery. The results showed that scanning patients’ knees with $^{18}$FFEPPA was able to accurately measure their knee inflammation. This scanning method could allow researchers to find new ways to treat OA by coming up with new drugs that can reduce inflammation in the knee and reverse the course of the disease. Researchers may also be able to use this scan to find connections between inflammation in joints and our gut health or the development of stiff joints as we age.
Co-Authorship Statement

The following thesis contains one manuscript that is in preparation for submission to publication (Chapter 2). The study was co-authored by Drs. Brent Lanting, Matthew Teeter, Thomas Appleton, and Jonathan Thiessen. This study was designed by Drs. Matthew Teeter and Brent Lanting. As the first author on this manuscript, I was a significant contributor to all aspects of the study and manuscript preparation. Specific responsibilities included all applications for ethics and regulatory approval before the study could commence. Then I was responsible for patient screening/recruitment. I oversaw all data collection, performed image segmentation/processing, and analyzed/interpreted all the data. I was responsible for drafting this thesis document with support from Dr. Thomas Appleton’s lab for immunofluorescence and histology experiment procedures. Dr. Jonathan Thiessen provided insight and guidance on PET/MRI imaging and oversaw the clinical imaging aspect of the study.
Acknowledgments

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To my advisory committee, Drs. Lanting, and Hicks. Thank you for guidance, providing my research with focus and direction. Thank you Dr. Lanting for your assistance with tissue collection in the operating room that has made my clinical research possible.

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I want to acknowledge Adam McIntyre for your flexibility on TSPO genotyping. Your blood result turnaround time helped me with tight imaging schedules. Heather Biernaski, Thanks for your assistance with the clinical PET/MRI scans at Lawson. It was great to work with you.

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Chapter 1

1 Introduction

1.1 Osteoarthritis

Osteoarthritis (OA) is a degenerative joint disease marked by loss of joint cartilage integrity and underlying bone remodeling. The prevalence of OA is increasing, mainly due to increased life span and rising rates of obesity. Rates of OA in Canada have been estimated at 14.2% across the entire Canadian population and up to 30% in those aged 80 or older. Knee OA is the most common type of osteoarthritis and is the cause of significant disability and reduced quality of life. Symptoms of OA are typically brought on by joint tissue changes leading to loss of cartilage. The loss of articular cartilage may start as a focal lesion but eventually extends to a progressive thinning across the whole surface. Superficial fibrillation on the surface of the articular cartilage is caused by the cleavage of proteoglycans in the cartilage extracellular matrix (ECM) by collagenases and aggrecanases. The degradation of cartilage in knee OA involves degradation of the ECM exceeding synthesis leading to a net decrease in cartilage. The primary enzymes thought to be responsible for this cartilage breakdown are matrix metalloproteinases (MMP) and a disintegrin with a metalloproteinase and thrombospondin motif (ADAMTS). The expression of several MMPs have been shown to be high in the cartilage of patients with end stage OA. Symptoms of the disease, like pain and stiffness, often present to clinicians late in the disease when structural changes have progressed for decades. Therefore, there is a need to study the pathology of OA at an early stage when inflammation initiates in response to disease conditions.

1.2 Disruption of All Joint Tissues

Despite recent advancements in treating the symptoms of the disease, biologic treatments to modify the course of the disease have remained elusive. Treatments to reduce pain and stiffness of knee OA include anti-inflammatory steroid injections, manipulation of
the joint under anesthesia, and synovectomy. In the most severe cases of treatment resistant OA, the joint is removed surgically and replaced with metal and plastic artificial implant components in a total knee arthroplasty (TKA)\textsuperscript{10}. These treatment options are relatively successful but not ideal in that patients live symptomatic for years while treating the disease and often suffer with reduced quality of life.

It would be preferable to modify the course of the disease early on through pharmaceutical interventions. Large portions of medical research have been devoted to developing interesting biologics to treat OA, however, at this point none have been successful in passing clinical trials.

The most common long-standing hypothesis of idiopathic OA is that it develops because of a continuous mechanical “wear and tear” of the joint\textsuperscript{11}. We now know that loading of the joint through regular, moderate exercise is protective of articular cartilage, possibly through the suppression of endoplasmic reticulum stress and promotion of autophagy of pathologic tissue\textsuperscript{12}. The knee joint is a complex system of tissues that work in concert to contribute to the joint’s overall functionality. A complete understanding of OA will involve an understanding of all joint tissues including articular cartilage, bone, ligament, tendon, menisci, fat pad, and the synovium (Fig. 1). OA involves dysregulation and derangement of all joint tissues and chronic inflammation plays a role in the disruption of joint tissue homeostasis\textsuperscript{13}.
Cartilage is the smooth, lubricated, surface that allows for smooth motion of bone over bone. It is avascular and aneural and consists of a network of extracellular matrix (ECM) rich in type II collagen to provide the load bearing properties to the tissue. Within the collagen matrix is a complex network of proteoglycans bound to hyaluronic acid that provide a smooth articulating surface for the joint. The cartilage is critical to healthy joint function and in healthy tissue chondrocytes regulate the structure of the tissue properly. Under OA conditions, signaling molecules such as interleukin (IL) and tumor necrosis factor (TNF) cause the release of degradative enzymes MMP and ADAMTS. Cleavage of key cartilage aggregates occurs, and integrity of the cartilage surface is compromised.
Inflamatory signaling conditions in OA also lead to a loss of homeostatic chondrocytes and an altered, hypertrophic, phenotype that promotes degradation and vascularization of the cartilage\textsuperscript{14}.

The subchondral bone plays a critical role in providing structure to the joint. The bone contains a mineralized ECM of type I collagen and interfaces with the cartilage. An important feature of OA is the signaling of bone remodeling. The cause of increased bone remodeling in early OA is unknown but some proposed mechanisms are increased bone-cartilage crosstalk, vascular invasion into the tissue, and signaling pathways like transforming growth factor β, insulin like growth factor, and wnt which are stimulators of osteoblast differentiation and were found to be upregulated in OA diseased osteoblasts\textsuperscript{15}. Local sites of increased bone remodeling create boney outgrowths called osteophytes. A hallmark of OA is the appearance of osteophytes and the vascularization and pain that are associated with this tissue remodeling.

1.3 Synovitis and Inflammatory Regulation

The synovium plays a critical role in the pathology of inflammatory joint conditions. The healthy knee synovium contains a 1-2 cell thick layer of mainly immune cells called the intima with a relatively acellular sub-lining layer. The function of the normal synovium is to facilitate transport of nutrients and waste between the joint and outside environment and to maintain joint homeostasis through cell signaling cytokine release by synovial tissue\textsuperscript{16}. Chronic low-level synovitis disrupts the normal function and structure of this critical tissue. Diseased synovium is marked by hyperplasia of the lining, increased vascularity, and inflammatory cell infiltration into the synovial intima\textsuperscript{17}. Infiltration of macrophages into the synovium was shown to be common in early and end stage OA and an unresolved proinflammatory macrophage state continues to signal catabolic pathways IL-x and TNF-x that will eventually lead to cartilage breakdown\textsuperscript{18}.

Inflamatory cell dysfunction in knee osteoarthritis is a complex process involving many subsets of immune cells and pathways. The role of various immune cell types on the knee
tissue level microenvironment is unclear and is a growing area of study. Global gene expression studies in OA knee tissue samples have shown differences in proportion of M1 and M2 macrophages, activated dendritic cells, T cells, and neutrophils when comparing diseased to healthy synovial tissue\textsuperscript{19}. What is clear is that understanding the role of immune regulation on the pathology of OA will be important to alter the course of the disease.

1.4 Macrophages in the Synovium

The resident immune cell of the synovial lining is the macrophage\textsuperscript{16}. Macrophages are a key regulator of inflammation and are often described as an M1 or M2 phenotype. The state of activated macrophages depends on the local signaling environment such as Interferon-\(\gamma\) (IFN), TNF-\(\alpha\) and lipopolysaccharide (LPS)\textsuperscript{20}. M1 macrophages generally secrete pro-inflammatory cytokines that signal tissue break down while the M2 phenotype displays an anti-inflammatory profile that promotes tissue repair pathways\textsuperscript{21}. The M1/M2 dichotomy is now regarded as a simplification that represents the extreme poles of a spectrum of many discrete and rapidly changing macrophage phenotypes\textsuperscript{22–24}. Chronic unresolved activation of macrophages measured by increases in macrophage associated molecules and chemoattractant molecules that draw macrophages to infiltrate the tissue was shown to be associated with clinical outcomes in OA\textsuperscript{25}. The current understanding of the etiology of macrophage disfunction is that synovial macrophages respond to patterns of cartilage breakdown products and intracellular proteins from necrotic cells. The subsequent macrophage activation signals more cytokines that worsen the tissue degradation\textsuperscript{26}. It is unclear why this immune dysregulation occurs or why homeostasis is never achieved, and the inflammatory process is not resolved as it normally should.

Developing tools to image macrophages in vivo will be critical to advance the understanding of the role these macrophages play on the regulation of inflammation and development of OA. Much of the current work on studying inflammatory pathways and macrophage subtypes has been done ex vivo. While this research is vital to the
understanding of disease progression at the tissue level, it will be critical to develop imaging modalities that can study early inflammation in vivo.

1.5 Imaging Joint Structure and Macrophages

Many imaging modalities can be used to assess features of OA like synovitis, bone remodeling, and cartilage degradation. Radiography is still the most used modality to assess OA despite its limitations\textsuperscript{27}. Typical x-ray analysis of OA will measure joint space narrowing and osteophyte growth. The limitations of basic radiography are well documented. The traditional Kellgren and Lawrence (KL) grading scale used to rank OA progression may not be able to detect small, early tissue changes in OA knees\textsuperscript{28}. Traditional radiography has strengths in cost, speed, accessibility, and ease of use; however, other limitations are the inability to assess soft tissue or biological activity of cells within the tissue.

1.5.1 Magnetic Resonance Imaging of OA

MRI has the distinct advantage of offering unparalleled soft tissue contrast without using any ionizing radiation. MRI takes advantage of the hydrogen nuclei that are ubiquitous in human tissue. \textsuperscript{1}H nuclei possess a single proton that has a quantum property called spin. The nuclear spin can be thought of as a magnetic dipole moment possessed by every \textsuperscript{1}H nuclei. MRI scanners generate a static magnetic field throughout the bore of the scanner that aligns \textsuperscript{1}H nuclei spin with the direction of the applied field. To produce imaging contrast, a radiofrequency (RF) coil is placed around the structure of interest. By application of an RF pulse perpendicular to the applied magnetic field, nuclei absorb the energy of the pulse and are perturbed from the field vector. The relaxation of these nuclear spins produces an emittance of energy that is detected by a suitable tuned coil. The unique environment of each \textsuperscript{1}H nuclei will produce a different relaxation characteristic when placed in the same magnetic field allowing for contrasting signal between different tissues.
There are two distinct processes of relaxation by which MR images are generated: Longitudinal relaxation ($T_1$) and transverse relaxation ($T_2$). $T_1$ is known as “spin-lattice relaxation” and is the length of time taken for the system to return 63% towards thermal equilibrium after an RF pulse. $T_1$ relaxation profiles can be manipulated by changing the time between pulse repetitions (TR) and the time between RF pulse and received echo (TE). In the context of orthopaedic imaging, $T_1$ weighted images can be produced where TR and TE are kept low, and the image is dominated by the $T_1$ properties of the tissues. Bone and fat will appear bright on $T_1$ weighted images. It is for this reason that $T_1$ weighted images are considered inadequate for imaging OA pathology as cartilage and effused tissue will remain dark. Conversely, $T_2$ weighted images are optimal to study the pathology of OA. TR and TE are set high, and the contrast is primarily determined by the $T_2$ properties of the tissue. Fluid filled structures like the synovium and cartilage will appear bright on $T_2$ weighted images. To distinguish fat from tissue pathology on $T_2$ weighted images, Short Tau Inversion Recovery (STIR) may be used among other fat suppression techniques to darken the relaxation range of fat tissue and provide better visualization of fluid structures.

In addition to standard MRI protocol, other pulse sequences such as 3D dual echo steady state (DESS) offer nearly isotropic, high-resolution scans. DESS scans produce images with negative bone and high fluid signal. This gives DESS ideal signal to noise ratio (SNR) when distinguishing bone, cartilage, and synovium. Typical MRI protocols acquire two dimensional (2D) fast-spin echo (FSE) sequences. While these images have excellent contrast and high in-plane spatial resolution, the out of plane resolution may not be optimal to visualize pathology due to the partial volume effect. 3D DESS minimizes partial volume effects and through plane distortion by acquiring thin and continuous slices that can be post processed to any desired imaging plane.

1.5.2 Positron Emission Tomography

Positron emission tomography (PET) offers unmatched imaging capability to target molecular and cellular activity that may even precede structural joint changes. The
strength of PET lies in the radioligands developed to target biological pathways. There are many commercially available PET tracers that can be used to image musculoskeletal disorders. These tracers involve a targeted ligand that will bind a molecule of interest in vivo, which is attached to a radioactive isotope that can be measured by a detector. When the radioligand is injected into the body it will preferentially bind the target. The decay of the radioactive isotope produces a positron through $\beta^+$ decay which quickly annihilates with a nearby electron. This annihilation event produces two co-linear photons with a signature energy level of 511 keV. A PET detector will amplify the photons using a scintillator crystal array and measure them with a photosensor. The co-linear property of these annihilation photons allows the PET detector to accurately localize the decay event within the FOV.

The use of PET tracers to assess musculoskeletal disorders can be broadly categorized into oncologic and non-oncologic applications. For this thesis, we will focus on non-oncologic applications of PET imaging. OA is a complex disease and the understanding of the causes of this disease have been supported by reliable non-invasive tools to detect early changes. Proven tracers like $[^{18}\text{F}]$fluorodeoxyglucose (FDG), a glucose analog, and $[^{18}\text{F}]$sodium fluoride (NaF) have been used in some limited MSK applications (Table 1). $[^{18}\text{F}]$NaF provides a method to assess subchondral bone calcium uptake, indicating changes in focal bone remodeling which had been implicated as a mechanism in OA progression. Studies in OA patients showed higher uptake of $[^{18}\text{F}]$NaF in hip joints that showed bone abnormalities through MRI. Uptake of $[^{18}\text{F}]$NaF in the patellofemoral compartment has also been shown to correlate with patellofemoral pain.

$[^{18}\text{F}]$FDG is a very common PET tracer that images upregulated glucose metabolism. Pathology in the body often comes with increased energy demands and $[^{18}\text{F}]$FDG can be useful to image this process. $[^{18}\text{F}]$FDG has been used to study the role of inflammation in OA, however, it is not specific to inflammation and only reveals areas of increased
glucose uptake. More targeted tracers have been developed to study inflammation directly.

$^{[18}F]$FEPPA is a third generation PET tracer that can target over expression of TSPO on activated macrophages. It is composed of a phenoxyarylacetamide based targeting moiety conjugated to a fluorine 18 radioisotope (Fig. 2). $^{[18}F]$FEPPA has typically been used to target activated microglial cells as a method to image brain inflammation. TSPO targeting tracers have shown increased uptake in patients with Alzheimer disease, Parkinson disease, and multiple sclerosis, indicating the potential use for $^{[18}F]$FEPPA in the pathology of brain inflammatory conditions$^{35-37}$. However, activated immune cells like macrophages overexpress TSPO in the synovium and $^{[18}F]$FEPPA may be a suitable target for imaging inflammation in joint structures. There have been some studies using earlier generation TSPO targeting tracers such as $^{[11}C]$PK11195 and $^{[11}C]$PBR28 to study inflammatory mechanisms in RA and some early OA pilot applications$^{38,39}$. These studies used older generation TSPO targeting tracers with suboptimal pharmacokinetic properties and were limited by very small sample sizes (n=1).
Figure 2: Chemical structure of $[^{18}\text{F}]\text{FEPPA}$.

Table 1: PET tracers used to assess musculoskeletal disorders.

<table>
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<th>Target</th>
<th>MSK Application</th>
<th>Half Life (m)</th>
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<tr>
<td>$[^{18}\text{F}]\text{FEPPA}$</td>
<td>Activated macrophages</td>
<td>Imaging active inflammation</td>
<td>109.8</td>
</tr>
<tr>
<td>$[^{18}\text{F}]\text{FDG}$</td>
<td>Glucose metabolism</td>
<td>Tumor staging, bone metastasis, infection</td>
<td>109.8</td>
</tr>
<tr>
<td>$[^{18}\text{F}]\text{NaF}$</td>
<td>Calcium uptake</td>
<td>Bone remodeling</td>
<td>109.8</td>
</tr>
<tr>
<td>$[^{18}\text{F}]\text{FTC-146}$</td>
<td>Sigma-1 receptor</td>
<td>Neuropathic pain</td>
<td>109.8</td>
</tr>
<tr>
<td>$[^{11}\text{C}]\text{Choline}$</td>
<td>Cell proliferation</td>
<td>Synovium proliferation</td>
<td>20.4</td>
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Designing novel PET tracers involves developing a suitable targeting ligand and choosing the appropriate radioisotope. Factors like the half-life, positron range, and chemical reactivity must all be considered when choosing the appropriate isotope for radiolabeling. Some advantages of using an isotope with a shorter half-life, like $^{11}$C, are the ability to image multiple times in the same day and a reduction in received radioactive dose for the same injected dose. However, logistical problems arise when half-life gets too short. For example, $^{11}$C has a half-life of 20.4 minutes and is relegated to use in imaging facilities with onsite cyclotron production. Any attempt to transport the tracer off site would lead to a tracer that had decayed too much by the time it could be used. It is also preferable to match the half-life of the isotope to the pharmacokinetics of the targeting molecule. Tracers like $^{11}$C, $^{18}$F, and $^{68}$G have shorter half-lives that are suitable for labelling smaller molecules and peptides that are cleared rapidly from circulation, in the order of minutes to hours. Longer lived isotopes like $^{89}$Zr (half-life: 78.4 h) would be more suitable to radiolabel molecules that persist in circulation for days. Producing a suitable targeting ligand for a PET tracer involves considerations of specificity to the target vs off target binding, clearance from non-target tissues, toxicity, and ability to reach target tissues. $[^{18}$F]FEPPA characterization has shown it to be preferential to earlier generation TSPO targeting ligands in terms of its clinical applicability, suitability to cross the blood brain barrier (lipophilicity), and high specificity for the target (TSPO).

1.5.3 Hybrid PET/MRI for Musculoskeletal Imaging

While MRI as described earlier has become the benchmark for MSK imaging due to the ability to non-invasively evaluate soft tissue, articular cartilage, and osseous structures, MRI is limited in the ability to image biological processes that are likely to precede structural changes. In contrast, PET offers sensitive ability to assess molecular and metabolic activity but lacks soft tissue contrast. Therefore, it is a natural pairing to combine the two imaging modalities and gain the strengths of both. PET is most often
combined with computed tomography (CT) imaging for its ability to provide density information and attenuation correction for PET photons. While PET/CT is essential for evaluation of MSK oncology, its utility is overshadowed by the unparalleled soft tissue contrast of MRI and lack of ionizing radiation.

New integrated PET/MRI systems provide a stand-alone and complete MSK imaging solution with high spatial resolution, excellent soft tissue contrast, and attenuation correction for PET photons using a two-point Dixon method\textsuperscript{43}. The limitation of these PET/MRI systems is the cost which may be greater than a standard integrated PET/CT machine.

High resolution 3D DESS images can be used to easily segment joint structures to assist in PET quantification. Segmentation of the suprapatellar synovium can be used to draw \[^{18}\text{F}\]FEPPA uptake statistics from fused PET/MRI images (Fig. 3). Then standard uptake values (SUV) can be calculated for the suprapatellar synovium which standardizes the observed \[^{18}\text{F}\]FEPPA signal for different patient weights and injected tracer doses. The synergy of PET to measure biological activity and MRI to correct attenuation and visualize soft tissue for segmentation will be useful for musculoskeletal research.
1.6 Autoradiography to Assess Macrophage Activation

Digital autoradiography is a high-resolution imaging method for localization of radiolabeled biomarkers ex vivo. Tissue samples should be prepared by fixation in formalin and embedded in paraffin. Then the tissue samples are sliced using a microtome between 5-20 µm. An appropriate concentration of $[^{18}\text{F}]{\text{FEPPA}}$ should be chosen so that the expected decay falls within the detection limits of the machine. Dilution of $[^{18}\text{F}]{\text{FEPPA}}$ may be required. Then the tissue can be fully submerged in tracer and
allowed to incubate. After sufficient uptake, the tissue is rinsed thoroughly and then placed in the wells of the machine (Fig. 4) and sealed in with a gaseous scintillator-coupled β-emission detecting camera. A charge is applied to the tissue sample and charged β-emission particles are accelerated to a detector where the uptake of tracer within the tissue can be quantified. Recent advances in digital particle counting over traditional phosphor screen autoradiography have allowed digital autoradiography to excellent sensitivity, dynamic range, and linearity with much lower data acquisition time45–47.

![Digital autoradiography sample holder for measuring positron decay in 18 standard glass slide wells.](image)

Any biologic molecule of interest, including drugs, metabolites, and cytokines, can be quantified efficiently in an ex vivo tissue sample using autoradiography48. This imaging
technique is useful to validate drug pharmacokinetics and distribution profile in tissues excised from animal disease models. In a previous study, $^{45}$C radioisotope was injected into terminally ill patients and autoradiography performed after death to assess short-term exchange of radiolabeled calcium on the bone surface$^{49}$. 

In the context of orthopaedic research, autoradiography can be used to assess biological activity in joint tissue. Ex vivo cartilage tissue studies showed that autoradiography can be used to assess proteoglycan deposition and synthesis in the articular cartilage ECM$^{50,51}$. Another study using radiolabeled annexin V, which preferentially binds to regions of collagen-induced arthritis, found that autoradiography could be used to assess cartilage changes in early rheumatoid arthritis (RA)$^{52}$. The work in this thesis will use autoradiography to assess macrophage activity in knee synovial tissue from patients with end-stage OA.

1.7 Imaging Validation with Immunofluorescence

Immunofluorescence (IF) is a widely used laboratory method in assessing pathology, cell classification, and diagnosis of disease. IF involves specific antigen-antibody reactions and has advantages over traditional tissue staining methods by targeting only a specific protein, enzyme, or tissue$^{53}$. Tissue to be analyzed is usually fixed in a formalin solution and then embedded in a block of paraffin wax allowing easy tissue sectioning to micrometer thickness$^{54}$. Tissue sections are then put through an antigen retrieval pretreatment to retrieve antigens masked by fixation of the tissue and make them more accessible for antibody binding$^{55}$. This process involves breaking cross linked proteins by either heat, ultrasound, or enzyme degradation. The primary anti-body to be used is diluted to an appropriate concentration so that the range of possible fluorescent intensities at each voxel will not exceed the detector’s limit. This optimization step is critical to avoid signal wash out. Tissue is incubated in one or more fluorescent antibodies and washed thoroughly. The stained tissue is imaged under confocal microscope which uses a spatial pinhole to block out of focus light and a photomultiplier to detect in plane light with great sensitivity. This allows for excellent optical resolution. Multiple imaging
channels can be created for each antigen-antibody binding profile when the fluorescent properties of each fluorophore are known. The result is a high resolution, sensitive image of multiple coloured imaging channels that represent the spatial distribution and concentration of antibody binding to the tissue.

1.7.1 Immunofluorescence in Assessing Osteoarthritis

IF plays a key role in the study of joint tissue disease. Changes to articular cartilage that are a hallmark of OA progression can be visualized by staining critical components of the tissue structure like collagen and proteoglycans. Collagen fibrillation can be visualized by fluorescent IF labelling and could be distinguished from proteoglycans, which coexist in the same tissue and share the same index of refraction\textsuperscript{56}. Another study used an antibody specific to type II collagen that has undergone oxidative post-translational modification to detect early pathogenic changes in articular cartilage\textsuperscript{57}. Synovitis and the characterization of OA stage can be assessed using IF. Ostoljic et al. showed that nuclear factor (NF-\textkappa B) and inducible nitric oxide synthase (iNOS), which is involved in the immune response of producing nitric oxide as an immune defense, were upregulated in synovial tissue of both early and late-stage knee OA patients\textsuperscript{58}.

IF is a powerful tool to study the pathology of early OA changes in all joint tissues, however, it is limited by the invasive nature of requiring a tissue sample. It is not feasible to require a sample when designing a research study. This is why non-invasive techniques will be critical to study early OA pathology when patients may not present clinical symptoms yet and tissue cannot be obtained.

For the purposes of this thesis, IF will be used as a gold standard validation method to compare statistical correlation between autoradiography and clinical scans to validate the accuracy of these other methods. A fluorescent probe and two antibodies were chosen in this assessment:

**DAPI** – a blue, fluorescent probe that targets the minor groove on double stranded DNA. Upon binding to the substrate, fluorescence is increased 20-fold and allows for the
visualization of adenosine-thymine rich regions of DNA. For this research, it was applied to locate cell nuclei as an indicator of cell location during image analysis.

**CD68** – a type 1 transmembrane glycoprotein with a suggested role in peptide transport or antigen processing. This protein is highly expressed in cells of the mononuclear phagocyte lineage including macrophages. It is widely used as an accepted indicator for macrophage cells.

**TSPO** – This antibody was used to target TSPO within the tissue. Quantifying this antibody binding concentration allowed for validation of the actual macrophage activity within the diseased tissue (Fig. 5).

Figure 5: Example of a confocal image of knee synovial tissue from a patient with end-stage OA. The tissue was stained with DAPI (blue), CD68 (red), and TSPO (green) antibodies.
1.7.2 Histology and Tissue Fibrosis

Fibrosis is a pathological feature of most chronic inflammatory conditions. It is defined by the accumulation of excess extracellular matrix components, mainly collagen. Joint tissues stiffen in response to excessive accumulation of collagen rich connective tissue resulting in reduced range of motion and pain during joint movement\(^5^9\). Articular cartilage is avascular and lacks the ability to repair itself so chondrocytes in a pro-inflammatory environment can undergo a conversion into fibrotic chondrocytes and begin to secrete proteins that are like fibrocartilage, which is stiffer and mechanically inferior to the intended hyaline cartilage\(^6^0,6^1\). Inflammatory initiation of fibrotic processes is not just limited to changes in articular cartilage. The synovium experiences excessive fibroblast proliferation and an imbalance between collagen synthesis and breakdown during periods of dysregulated synovitis\(^6^2,6^3\). In patients with OA, the synovium becomes thicker and more rigid\(^6^4\). These symptoms contribute to the typical symptoms of OA, such as joint pain and stiffness\(^6^5\). While inflammation is observed more in early stages of OA, it has been reported that there is more fibrosis in late-stage OA, suggesting an inverse relationship between inflammation and fibrosis\(^6^6,6^7\).

Dysfunction of inflammatory processes contribute to the etiology of joint fibrosis through the secretion of pro-inflammatory cytokines by synoviocytes. One of the most important pathways in this process may be transforming growth factor beta (TGF\(\beta\)) which works as a positive mediator that promotes ECM protein synthesis in cartilage. Animal models of OA show that TGF\(\beta\) upregulation induces synovial fibrosis\(^6^8,6^9\).

Hematoxylin and Eosin (H&E) staining is the most widely used method for assessing tissue samples. H&E staining has been validated to assess synovial tissue in RA samples with synovitis\(^7^0,7^1\). The tissue is fixed in formalin and paraffin embedded, then sectioned and placed on a glass slide. Incubation with Hematoxylin reveals ribosomes and chromatin in purple and Eosin shows cytoplasm, collagen, and connective tissue in orange (Fig. 6). The combination of these stains and a bright field microscope allows for visualization of pathology in many tissue types. Synovial tissue can be stained this way
and graded for 6 components that are key to the pathology of OA, synovitis, and fibrosis including: synovial lining thickness (hyperplasia), synovial cell infiltration, fibrin deposition, vascularization, fibrosis, and perivascular edema. H&E staining can also reveal important microscopic tissue changes in OA. Surface changes and disruptions that are common in OA can be visualized as undulations, fissures, or fibrillations in the normal smooth outer layer of the synovial tissue. Thickening of the synovial lining and infiltration of cells into the sub lining can also be detected (Fig. 6).

Figure 6: Example of knee synovial tissue from a patient with end-stage osteoarthritis that has been stained with H&E for histological grading.

1.8 Patient Reported Outcome Measures

Patient reported outcome measures (PROMs) are given to patients to quantify their subjective levels of pain, joint function, and quality of life. In the context of orthopaedics, patients are given PROM questionnaires before and after TKA to assess patients in these areas. European Quality of Life (EQ-5D-5L), Veterans RAND 12 (VR-12), University of
California: Los Angeles Activity Score (UCLA), Western Ontario and McMaster Arthritis Index (WOMAC), Knee Society Score (KSS), and Oxford Knee Score (OKS). Are all questionnaires that can be administered to assess these outcomes.

**EQ-5D-5L** – A 5-dimensional health questionnaire that determines the patient’s weighted value of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. EQ-5D-5L has been widely used and tested on both general population and patient samples.⁷³

**VR-12** – One of the most used global health measure questionnaires. VR-12 is a 12-point instrument meant to assess general health and QoL.⁷⁴

**UCLA** – The UCLA Activity Score is a questionnaire assessing physical activity level from 1 (low) to 10 (high) primarily used in patients undergoing hip or knee arthroplasty.⁷⁵

**WOMAC** – A disease specific instrument meant to assess the clinically important changes in health status because of treatment intervention in OA patients. WOMAC measures pain, stiffness, and physical function.⁷⁶

**KSS** – The Knee Society Score was developed as a simple but objective way to measure a patient’s functional abilities such as walking or climbing stairs to be assess before and after TKA.⁷⁷

**OKS** – Similar to KSS, the Oxford Knee Score is a 12-item patient reported instrument to assess function and pain after TKA. It is a short questionnaire but reproducible, valid, and sensitive to clinically important changes after surgery.⁷⁸

PROMs are a simple and cost-effective method for quantifying clinically relevant features of OA, however, they come with limitations. There may be difficulty distinguishing subtle differences between the extreme low and high values in a questionnaire. This is called the floor and ceiling effect and may suggest limited instrument range, measurement accuracy, and response bias when patients select the lowest or highest possible response.⁷⁹ Additionally, PROMs are inherently subjective in
nature and are greatly influenced by patient factors like pain, expectations, and catastrophization\textsuperscript{80}.

1.9 Thesis Objectives and Hypothesis

The primary objective of this thesis is to (1) validate the use of \([^{18}\text{F}]\text{FEPPA}\) in the assessment of macrophage activity in knee synovial tissue. The secondary objective is to (2) look for associations between macrophage activity measured by \([^{18}\text{F}]\text{FEPPA}\) uptake in knee synovial tissue and other important clinical features of OA like pain, stiffness, and knee function.

We hypothesize that \([^{18}\text{F}]\text{FEPPA}\) uptake in knee synovial tissue measured \textit{ex vivo} through autoradiography and \textit{in vivo} through PET/MRI will be correlated to the true macrophage activity of that tissue measured through immunofluorescence TSPO antibody staining. Additionally, we hypothesize that there will be positive associations between \([^{18}\text{F}]\text{FEPPA}\) uptake in knee synovial tissue and pain and stiffness and a negative association with knee function.
Chapter 2

2 Quantification of Macrophage Activity in Knee Synovial Tissue Using [$^{18}$F]FEPPA PET Radioligand

2.1 Introduction

Osteoarthritis (OA) is a complex disease that is characterized by pathological changes across all knee-joint tissue types including, cartilage, bone, ligaments, menisci, and the synovial membrane\textsuperscript{81}. Inflammation of the synovial lining of the knee (synovitis) has a pathogenic role in the development of OA\textsuperscript{17,82,83}. Macrophages are the dominant immune cell in the knee synovium and regulate the inflammatory process through secretion of proinflammatory cytokines\textsuperscript{84}. Activated macrophages secrete cytokines, like IL-1β and TNF-α, which signal catabolic processes that break down cartilage, indicating a possible link between macrophage activity in the knee synovium and clinical symptoms of OA in other knee joint tissues\textsuperscript{85}. Inflammation is highly heterogenous between patients with OA and a better understanding of mechanisms of knee synovial macrophage dysregulation in the progression of OA is required\textsuperscript{86,87}.

Magnetic resonance imaging (MRI) has been used to characterize synovitis as well as cartilage degradation and bone remodelling\textsuperscript{88}. Although MRI can discern changes in soft tissue structures it lacks the ability to view the underlying biological processes that are relevant to OA pathology.

In contrast, positron emission tomography (PET) has been used to image metabolic and immune changes in joint disease\textsuperscript{89–91}. Several commercially available PET tracers have been used to study metabolic processes in OA. Sodium fluoride ([$^{18}$F]NaF) has been shown to probe bone remodelling\textsuperscript{92}. Fluorodeoxyglucose ([$^{18}$F]FDG) is a widely used marker for glucose metabolism and is useful to image acute cellular changes in OA\textsuperscript{93}. 
Imaging synovial proliferation as an indicator for synovitis has been proposed with choline ([\(^{11}\)C]Choline) and high uptake was seen in sites of arthritic change\(^94\).

Translocator protein (TSPO) is an 18kDa protein on the surface of mitochondria. The function of this protein is unclear but a role in steroidogenesis, apoptosis, and cholesterol transport have been proposed\(^95\). TSPO is over-expressed on the surface of activated macrophages and has been increasingly popular as a target for imaging active inflammation\(^96\). TSPO targeting PET tracers have been used to examine the etiology of rheumatoid arthritis\(^97\). [\(^{18}\)F]FEPPA is a 2\(^{nd}\) generation radiopharmaceutical that preferentially binds TSPO and can be used to image activated macrophages\(^98\). Current applications of [\(^{18}\)F]FEPPA in PET imaging have been limited mainly to imaging brain glial cell inflammation\(^99\).

Hybrid [\(^{18}\)F]FEPPA PET/MRI may enable the potential to understand the early inflammatory and structural changes associated with the complex disease process of OA\(^100,101\). Therefore, this study aims to validate the use of [\(^{18}\)F]FEPPA radioligand in the quantification of macrophage activity in knee synovial tissue and to investigate the feasibility of using [\(^{18}\)F]FEPPA PET/MRI in the assessment of synovitis in vivo.

2.2 Methods

2.2.1 Study Design

This study consisted of a cohort of 6 participants that were recruited prospectively from the Rorabeck-Bourne Joint Replacement Clinic (University Hospital, London, Ontario, Canada) to take part in tissue autoradiography, IF, and a clinical PET/MRI scan (Fig. 7). A subgroup of 6 participants was gathered retrospectively from the Western Ontario Registry for Early Osteoarthritis (WOREO), a study designed to investigate the associations of inflammation in patients with knee OA. Patients from this subgroup had suprapatellar synovial tissue previously collected and only autoradiography and IF were performed.
2.2.2 Prospective Participant Eligibility Requirements

This study was approved by Western University Health Science Research Ethics Board (REB #118545) and Lawson Health Research Institute (Lawson R-21-506). Authorization to manufacture radiopharmaceuticals was given by Health Canada and approval to administer $[^{18}F]$FEPPA as described in this study was given by the Human Radionuclide Safety and Scientific Review Committee. All participants provided informed written consent at the time of enrollment in this study.

The primary inclusion criteria were a diagnosis of end-stage OA requiring a unilateral total knee arthroplasty. Exclusion criteria were patients who had inflammatory arthritis or a previous infection in the index knee. Patients who had undergone a prior knee surgery were also excluded. Contraindications for PET and MRI including pregnancy and pacemakers were exclusions for this study.

$[^{18}F]$FEPPA offers high specific signal and robust quantification but like many other TSPO targeting radioligands, it is affected by a single nucleotide polymorphism in the TSPO coding gene (rs6971)$^{102}$. This polymorphism is a single nucleotide substitution from A to T at position 147 in the fifth transmembrane domain. The resulting steric hinderance of $[^{18}F]$FEPPA binding produces 3 distinct phenotypes: C-C (high), C-T (mid), T-T (low). Genotyping for this polymorphism was performed as previously described using peripheral whole blood$^{102}$. Participants were excluded from the study if they possessed the low affinity binder (LAB) polymorphism. High affinity binder (HAB) and mid affinity binder (MAB) were included in this study. Participants that consented to the study and had blood drawn for rs6971 polymorphism analysis were composed of 75% HAB (6/8), 12.5% MAB (1/8), and 12.5% LAB (1/8) genotypes. The rates of rs6971 genotypes in the population vary with ethnicity. People of European ancestry are more likely to possess the LAB genotype. A database of polymorphism information lists the rate of rs6971 genotypes in North America to be 71.8% HAB, 25.9% MAB, and 2.3% LAB$^{103}$. 
Figure 7: Flowchart of study activities for participants recruited prospectively from the Rorabeck-Bourne Joint Replacement Clinic for PET/MRI imaging.

2.2.3 Retrospective Participant (WOreo) Eligibility Requirements

All Participants provided informed consent and the study was approved by Western University’s Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB #109255). Demographics including age, sex, and body mass index were collected (Table 2).
The primary inclusion criteria for these participants were symptomatic, late-stage knee OA with a KL grade of 3 or 4 who were scheduled to undergo a TKA. Participants were excluded if synovial histopathology data was not available.

Table 2: All participant demographics

<table>
<thead>
<tr>
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<th>Prospective (n=6)</th>
<th>Retrospective (n=6)</th>
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</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>68.5 ± 4.4</td>
<td>69 ± 5.7</td>
</tr>
<tr>
<td>Sex</td>
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<td>3 male, 3 female</td>
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<tr>
<td>Height (cm)</td>
<td>168.5 ± 11.9</td>
<td>170.3 ± 8.6</td>
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<tr>
<td>Mass (kg)</td>
<td>83.4 ± 21.0</td>
<td>91.5 ± 13.1</td>
</tr>
<tr>
<td>BMI</td>
<td>29.0 ± 5.28</td>
<td>31.8 ± 5.5</td>
</tr>
</tbody>
</table>

BMI, body mass index

2.2.4 Synovial Tissue Preparation

Fixation and Paraffin Embedding

During the participant’s TKA the surgeon acquired synovial fluid and tissue samples. Tissue samples were fixed overnight at 4 C in 4% paraformaldehyde then transferred to a 70% ethanol solution until being run through a tissue processor to dehydrate and imbue the tissue with paraffin wax. The tissue was embedded in paraffin wax and 5 µm sections were collected on slides using a microtome. Slides were baked at 60 C for 25 minutes and then cooled to room temperature. Sections were then deparaffinized using xylene and rehydrated with decreasing concentrations of ethanol (100%, 95%, 70%, 0%)
Cryopreservation

Fixed synovial tissue was bathed in 20% sucrose solution for 48 hours and then 40% sucrose solution for another 48 hours at 4 C. Tissue was cryopreserved in optimal cutting temperature compound (OCT) (Fisher Scientific, 23-730-571) by rapidly freezing on dry ice and stored at -80 C. Tissue temperature was equilibrated for 30 minutes until -25 C and 2 serial sections per slide were cut to 8µm thickness using a cryostat (Leica CM1950). Samples were mounted on Superfrost Plus slides (Fisher Scientific, 12-550-15) and stored at -80 C until needed.

2.2.5 Autoradiography

Cryosectioned tissue was allowed to thaw to room temperature and then washed for 15 minutes in a submersion of phosphate-buffered saline (PBS – 137mM NaCL, 2.7mM KCl, 10mM Na₂HPO₄, 1.8mMKH₂PO₄). Sections were incubated in PBS solution containing 50kBq of [¹⁸F]FEPPA for 1 hour for adequate tissue uptake. The slides were washed with ice cold PBS for 60 seconds followed by a final 60 second wash with ice cold distilled water. Finally, the slides were allowed to air dry and placed in a digital autoradiograph (BeaQuant Beaver JTV-18-0009). Images were gathered over 6 hours with an image size of 23.04cm². Each pixel was 100µm² giving an image resolution of 4800x4800 pixels.

After all scan data was gathered, tissue sections were incubated in Coomassie brilliant blue (Fisher Scientific) to visualize the dense synovial intima to aid in image segmentation. Tissue sections were incubated for 5 minutes in 100% Coomassie brilliant blue followed by a 1-minute wash in tap water. Sections were allowed to air dry and then imaged on a compound lens microscope at 40X magnification (Leica, DM4 B). Stained tissue and autoradiography images were scaled and fused for intima segmentation.

 Autoradiography images were segmented with ROI defined as the synovial intima (Beaquant, Beamage 3.0). The mean particle count per square millimeter was calculated as the measure of [¹⁸F]FEPPA uptake concentration in the tissue.
2.2.6 Immunofluorescence

Cryosectioned tissue was removed from the freezer and allowed to thaw to room temperature. Slides were hydrated in 1X PBS for 20 minutes. The glass slides were blotted dry, and a hydrophobic ring drawn around each tissue section to create a barrier for antigen retrieval. Heat mediated antigen retrieval was performed by microwaving sodium citrate buffer (pH=6.0) to 95 C. Slides were submerged and allowed to cool to room temperature. Tissue was permeabilized with 0.2% Triton-x 100 in 1X PBS for 10 minutes. Then the tissue sections were washed 3 times with 1X PBS for 2 minutes each. Tissues were blocked with 1X PBS containing 5% bovine serum albumin (BSA) and 0.1% Triton-x 100 for 1 hour at room temperature.

Primary antibodies were applied to one section and isotype control antibodies to the other section and incubated overnight at 4 C. Primary antibodies used were anti-TSPO (Abcam, ab109497) 1.9µg/ml and anti-CD68 (Abcam, ab201340) 0.4µg/ml. Isotype controls for any nonspecific immunoglobulin binding that may occur were rabbit IgG isotype control (Abcam, ab171870) 1.9µg/ml and mouse IgG isotype control (Abcam, ab170190) 0.4µg/ml. The next morning slides were washed 3 times in 1X PBS for 2 minutes each.

Secondary antibodies were applied to each section and incubated for 1 hour at room temperature and protected from light. Goat anti-rabbit IgG alexa 488 (Jackson Immunoresearch, 111-545-144) and goat anti-mouse IgG alexa 647 (Jackson Immunoresearch, 115-605-003) were used. Sections were washed 3 times in 1X PBS for 2 minutes each. Sections were mounted with prolong antifade DAPI (Fisher Scientific, P36931) and cured for 24 hours protected from light.

Images were acquired on a confocal microscope (Leica SP8 confocal). DAPI images were gathered using a HyD detector with a gain of 10%. 488 was imaged using a PMT detector with a gain of 450V and 647 was imaged using a PMT detector with a gain of 600V. The images were gathered with a resolution of 2048x2048 pixels, with a frame
average of 2 and a z slice thickness of 0.5µm. Max projection images were produced with identical settings on LAS-X built-in software to ensure equivalence for comparison.

Images were opened in ImageJ 1.53t (National Institutes of Health, USA) for processing. Multi-channel images were segmented with a ROI defined as each cell using the blue channel (DAPI) as a guide for the location of each cellular nuclei and localization of CD68 and TSPO to guide tracing of cell boundaries. Another set of ROIs was generated to include only cells with signal in the red channel (CD68+ only). A third set of ROIs was generated to include only cells with signal in both the red and green channels (CD68+ and TSPO+). Mean and max signal intensity in each imaging channel, as well as average segment area, was extracted for each set of ROIs.

2.2.7 Histology

Tissue sections were fixed and embedded in paraffin as described, but not cryosectioned for histology. Tissue from 5 slides representing every 100 µm distance along the tissue sample were stained with Harris Hematoxylin (Leica, 3801562) and Eosin Y (Fisher Scientific, E511) using the following protocol. The tissue was incubated in Harris hematoxylin for 5 minutes followed by a 5-minute rinse in tap water. Tissue was then dipped in 1X acid alcohol to remove hematoxylin from the cytoplasm followed by another 5-minute rinse in tap water. Another dip in 1X alkaline water was done to turn hematoxylin blue followed by a 5-minute tap water rinse. Eosin Y was incubated with the tissue for 5 minutes and then dipped in alcohol twice in 95% ethanol solution and twice in 100% ethanol solution to remove any weakly bound areas of Eosin. Lastly, tissue samples were dipped in xylene 30 times and mounted in xylene based mounting media (cytoseal) and a coverslip was placed on top.

The most damaged region of each slide was scored at 200X magnification on a scale of 0 (none) to 3 (severe) for each of six components including synovial lining thickness (hyperplasia), synovial infiltration, fibrin deposition, vascularization, fibrosis, and perivascular edema, and the median score of the 5 slides was used as described in
previous studies. The grader had been calibrated against an expert scorer with kappa values of 0.6 or above.

### 2.2.8 PET/MRI Protocol

Bilateral $^{18}$F FEPPA knee scans were performed on 6 of the recruited participants at Lawson Health Research Institute (St. Joseph’s Hospital, London, Ontario, Canada) using a 3T Siemens Biograph mMR hybrid imaging system (Siemens Healthineers, Knoxville, TN, USA). $^{18}$F FEPPA prepared for intravenous injection was manufactured at Lawson’s cyclotron (St. Joseph’s Hospital, London, Ontario, Canada). Up to 185MBq of $^{18}$F FEPPA was injected as an intravenous bolus followed by a 20ml saline flush. After a 45-minute tracer uptake period, participants were loaded into the bore of the scanner. A dual channel, body and spine, rf coil was positioned over the participants knees to allow simultaneous, bilateral, imaging. A list mode PET acquisition was started at the beginning of the MRI protocol. Two-point DIXON and ultra-short echo MRI acquisitions were gathered to segment water, tissue, fat, and air for MR-based metal artifact correction (MRAC) of the PET images. The MRI protocol included coronal T1 weighted images for MRAC, sagittal T2 weighted images for high quality images used to segment the supra-patellar synovium, and sagittal dual echo steady state (DESS) to distinguish cartilage from synovial fluid. Participants with a previous knee replacement in the contralateral knee had the MRI protocol modified to metal artifact reduction sequences (MARS) available in Siemens Advanced WARP package. These included 2D axial, sagittal, and coronal fast spin echo (FSE) images with view angle tilting (VAT) and 3D FSE images with slice encoding for metal artifact correction (SEMAC) and the short inversion time inversion-recovery (STIR) technique for fat suppression. In addition to metal correction protocol, MRAC μ maps were manually corrected using a method to inpaint the missing MR data created by metal artifacts using linear attenuation coefficient (LAC) values for tissue, bone, and available 3D implant models as validated in a cadaveric knee study by Bourdon et al. PET data sets were retro-reconstructed with manually corrected μ maps to correct for photon attenuation more accurately. MRI sequence parameters are included in Table 3.
Using 3D DESS or T2 FSE MRI, suprapatellar synovial volume was manually segmented using 3D slicer (www.slicer.org). MRI synovial segmentations were applied to the fused PET data to extract the signal intensity of \(^{18}\text{F}\)FEP\(\text{P}\) in the synovium (Fig. 3). Standard uptake value (SUV) for \(^{18}\text{F}\)FEP\(\text{P}\) was calculated using the mean signal intensity, body weight, and decay corrected injected dose.

**Table 3. MRI Scanning Parameters used for T1, T2 and 3D DESS scans.**

<table>
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<th>3D DESS index knee</th>
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<tr>
<td>(mm)</td>
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<tr>
<td>Slices acquired</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Acquisition matrix</td>
<td>256x256</td>
<td>256x256</td>
<td>448x448</td>
</tr>
<tr>
<td>Fat suppression</td>
<td>STIR</td>
<td>STIR</td>
<td>WE</td>
</tr>
</tbody>
</table>

Abbreviations: TE – echo time, TR – repetitions time, FA = flip angle, FOV = field of view, DESS – dual echo steady state, STIR – Short tau inversion recovery, WE = water excitation.

### 2.2.9 Statistical Analysis

All data was assessed for normality using the D’Agostino and Pearson omnibus normality test with a significance value of \(\alpha=0.05\). Depending on the normality of the data, Pearson or Spearman correlations were calculated. Correlations that would produce monotonic
relationships were analyzed with the non-parametric Spearman correlation ($\rho$) used when at least one variable is measured on an ordinal scale. Level of significance was set at $p < 0.05$. Pearson correlation and significance were calculated between the $[^{18}\text{F}]\text{FEPPA SUV}$ drawn from PET data and the macrophage activation determined from IF. All statistical tests were conducted using GraphPad Prism v9.5.1 (GraphPad Software, La Jolla, CA).
2.3 Results

2.3.1 Immunofluorescence

Fluorescence from DAPI bound to DNA was used to segment each cell in the synovial lining. Cell boundaries were traced using CD68 and TSPO localization as a guide (Fig. 8). Statistics were then drawn on segments that had fluorescent signal from CD68 and TSPO. The fraction of each cell type and the mean intensity of macrophage activation was derived from these segmentations.

Figure 8: Example of confocal microscope immunofluorescent image segmentation. Each cell was segmented using blue channel DAPI fluorescence and statistics were drawn on segments that co-expressed CD68 (red) and TSPO (green).
The percentage of cells within the synovial intima that were CD68$^+$ was 78.4% ± 17.5% (range, 46.1% - 100%). The proportion of CD68$^+$ cells that existed in an active state determined by co-staining of TSPO was 90.9% ± 11.4% (range, 68.4% - 100%) (Fig. 9).

Figure 9: Proportion of the cells in the synovial intima that were counted as showing immunofluorescent signal in TSPO, CD68, and DAPI (dark purple), CD68 and DAPI (light purple), and DAPI only (gray).

Mean TSPO fluorescent signal in the activated macrophages determined through segmentation of cells that were TSPO$^+$ and CD68$^+$ ranged from 2.36 – 19.82 ± 5.49 (Fig. 10). Samples 2 and 12 are missing from Figure 10 as it was determined that not enough synovial tissue was present among the tissue sample for analysis.
Figure 10: Average intensity per cell of fluorescent signal from TSPO antibody binding. Mean TSPO fluorescent intensity ranged from $2.36 - 19.82 \pm 5.49$. Error bars represent standard deviations in mean TSPO signal from each cell in the synovial tissue.

Immunofluorescent images showed a range macrophage activation represented by increased TSPO fluorescence in the green imaging channel. Sample 4 showed low macrophage activation, sample 5 had intermediate macrophage activation, and sample 9 showed the highest macrophage activation with high TSPO immunofluorescent signal present among nearly all cells within the synovial tissue (Fig. 11). Negative control staining was done on sample 10 and was incubated with only DAPI and secondary antibodies to visualize nonspecific binding. The signal in the red and green channels was 0 in the negative control indicating that the signal observed was from binding of primary antibodies and not nonspecific secondary antibody targeting. (Fig. 11).
Figure 11: Examples of confocal images taken of tissue stained with DAPI (blue), anti-CD68 (red), and anti-TSPO (green). Samples were chosen to represent a range of macrophage activation (green TSPO immunofluorescent intensity). Negative control was performed on sample 10 and incubated with DAPI and secondary antibodies only.

2.3.2 Autoradiography

 Autoradiography images generated are shown in Figure 12. Each discrete section of blue signal represents the uptake of $^{18}\text{F}$FEPPA indicated by measured $^{18}\text{F}$ positron decay.
Tissue segments were prepared on each slide in duplicate with adjacent tissue segments. The image was segmented and the mean particle count per minute per millimeter squared of tissue was used for statistical analysis.

![Autoradiography image of each synovial tissue sample](image)

**Figure 12**: Autoradiography image of each synovial tissue sample. Slides were prepared with duplicate adjacent tissue sections. Each section of tissue was segmented, and the average value used for statistical analysis.

Mean $[^{18}\text{F}]$FEPPA signal was $46.7 \pm 26.5$ counts of radioactive decay/min/mm$^2$ of tissue (range, 12.5 – 116.6). The mean tissue size was $6.0 \pm 4.9$ mm$^2$ (range, 0.36 – 18.6 mm$^2$)(Fig. 13)
Figure 13: Average $[^{18}\text{F}]$FEPPA uptake calculated from autoradiographic analysis of the synovial intima tissue. Spearman $r = 0.8545 \ (p = 0.0029)$.

$[^{18}\text{F}]$FEPPA uptake in knee synovial tissue measured ex vivo through autoradiography correlated significantly to antibody staining for TSPO (Fig. 14). The spearman correlation for this relationship was $r = 0.8545 \ (p = 0.0029)$. Samples 2 and 12 were omitted from this calculation due to insufficient synovial tissue present in the surgical sample. This resulted in 10 data points for correlational statistics.
Figure 14: Correlation between [\(^{18}\text{F}\)]FEPPA tracer uptake ex vivo and fluorescent TSPO signal from antibody staining.

2.3.3 PET/MRI

[\(^{18}\text{F}\)]FEPPA uptake was seen in the supratellar synovium of each participant. Representative high and low PET SUV are shown below (Fig. 15).
Some participants had large variation in [18F]FEPPA uptake between the index and contralateral knee (Fig. 16). This indicates a difference in macrophage activation between participants’ knees in some cases. Clinical reports of bilateral and unilateral OA were not gathered but may explain bilateral knee uptake variation.
Figure 16: T1 bilateral fused PET/MR image of participant 10’s knees in the coronal plane. Large variation in $[^{18}\text{F}]$FEPPA uptake between the index knee (left in the image) and contralateral knee (right in the image).

$[^{18}\text{F}]$FEPPA $\text{SUV}_{\text{mean}}$ in the index knees was $0.50 \pm 0.15$ (range, $0.22 – 0.68$). SUV$_{\text{mean}}$ in the contralateral knees was $0.35 \pm 0.18$ (range, $0.10 – 0.59$)(Fig. 17). Previous TKA implant components in the contralateral knee were present in participants 1 and 3. These two participants had the highest PET SUV in the contralateral relative to the index knee among all 6 participants. This suggests that the contralateral knee that had been diseased and treated through arthroplasty still exhibits signs of inflammation in the synovium.
Figure 17: $\text{SUV}_{\text{mean}}$ in the suprapatellar knee synovial segmentation for the patient’s index and contralateral knees. Previous contralateral TKA were present in participants 1 and 3.

There was a strong association between $\text{SUV}_{\text{mean}}$ calculated from $[^{18}\text{F}]\text{FEPPA PET/MRI}$ in the synovium and the intensity of immunofluorescent TSPO antibody staining in the same tissue (spearman $r = 0.90$, $p = 0.0833$)(Fig. 18).
Figure 18: Correlation between $[^{18}\text{F}]$FEPPA uptake in knee synovial tissue during PET scan and immunofluorescent TSPO antibody staining.

2.3.4 Histology

Tissue grading assessed through H&E stained histology is summarized in Table 4.
**Table 4: Histological values of important tissue-level OA features graded from 0 (none) to 3 (severe).**

<table>
<thead>
<tr>
<th>Osteoarthritis Component</th>
<th>Mean ± Standard Deviation (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovial lining thickness</td>
<td>0.75 ± 0.83</td>
</tr>
<tr>
<td>Synovial cell infiltration</td>
<td>1.1 ± 0.76</td>
</tr>
<tr>
<td>Fibrin deposition</td>
<td>0.83 ± 0.37</td>
</tr>
<tr>
<td>Vascularization</td>
<td>1.8 ± 0.72</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1.6 ± 0.86</td>
</tr>
<tr>
<td>Perivascular edema</td>
<td>0.67 ± 0.94</td>
</tr>
</tbody>
</table>

Tissue sample 7 was high in fibrotic build up and graded a 3 on fibrosis. Sample 8 and 9 were highly infiltrated by immune cells and scored highly on subsynovial infiltration. This is apparent by the density of stained cell nuclei beyond the outer layer of tissue. Sample 10 presented abnormal vascularization which can be seen as the abundance of circular vessels penetrating the tissue. Representative images of H&E-stained synovial tissue samples used for grading OA tissue features are shown below (Fig. 19).
Figure 19: Example H&E-stained synovial tissue samples used for histological grading of important osteoarthritis disease components.

There was no significant correlation between histological knee synovial tissue grading and autoradiography tracer uptake, IHC immunofluorescence, or PET SUV$_{\text{mean}}$ (Fig. 20).
2.3.5 Patient Reported Outcome Measures

Questionnaires answered before surgery about general health, quality of life, and knee function are shown below (Table 5). There was high variability in the scores reported in these health questionnaires.

Figure 20: Spearman correlation heat map between imaging methods and 6 components of histological OA tissue grading. Blue represents a positive relationship and red represents a negative relationship. The intensity of colouring is relative to the magnitude of the correlational strength.
Table 5: Patient reported outcome measures, presented as mean ± SD

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Mean Value ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCLA</td>
<td>3.6 ± 1.8</td>
</tr>
<tr>
<td>PCS</td>
<td>32.1 ± 9.5</td>
</tr>
<tr>
<td>VR12</td>
<td></td>
</tr>
<tr>
<td>MCS</td>
<td>42.3 ± 11.2</td>
</tr>
<tr>
<td>EQ-5D-5L</td>
<td>0.59 ± 0.16</td>
</tr>
<tr>
<td>Pain</td>
<td>56.2 ± 19.9</td>
</tr>
<tr>
<td>WOMAC</td>
<td></td>
</tr>
<tr>
<td>Stiffness</td>
<td>47.1 ± 20.9</td>
</tr>
<tr>
<td>Function</td>
<td>62.3 ± 13.7</td>
</tr>
<tr>
<td>OKS</td>
<td>26.1 ± 9.0</td>
</tr>
<tr>
<td>KSS</td>
<td>45.3 ± 10</td>
</tr>
</tbody>
</table>


The scores from PROMs and values of macrophage activation from experimental imaging were analyzed statistically with a Spearman correlation matrix to look for relevant associations between macrophage activation and clinically relevant symptoms of OA (Fig. 21). The only significant correlation was seen between the EQ-5D-5L questionnaire and TSPO immunofluorescence and Autoradiographic [18F]FEPPA uptake.
inflammatory processes are critical to tissue damage and wound repair pathways. This suggests a negative correlation as macrophage activation increases in knee synovial tissue the participants quality of life goes down. There were other trends of strong correlation between macrophage activation and PROMs but no other associations met statistical significance (p < 0.05).

![Spearman correlation matrix heat map of macrophage activation measured through experimental imaging and patient reported outcome measures.](image)

**Figure 21:** Spearman correlation matrix heat map of macrophage activation measured through experimental imaging and patient reported outcome measures.

### 2.4 Discussion

Recent evidence has suggested that the inflammatory process and mediators may play an important role in the initiation and progression of OA. Inflammation may not be a secondary feature of OA as once thought and it is important to recognize that inflammatory processes are critical to tissue damage and wound repair pathways. Tissue
damage is often mediated by inflammatory cytokines in the joint. An understanding of the role of inflammation in the progression of OA will be critical to develop disease modifying treatment. The synovium is a critical tissue in maintaining joint homeostasis and regulating inflammation. As the resident immune cell of the synovium, macrophages can be activated to mediate the inflammatory process and are important to study the pathology of OA.

We demonstrated in this study that activated macrophages could be imaged using $^{[18}\text{F}]\text{FEPPA}$ to target TSPO overexpression on activated macrophages. We observed a correlation between ex vivo autoradiographic $^{[18}\text{F}]\text{FEPPA}$ uptake and the true measure of macrophage activation measured by immunofluorescence ($r = 0.8545$, $p = 0.0029$). This suggests that $^{[18}\text{F}]\text{FEPPA}$ PET may be a useful tool to image activated macrophages in knee synovial tissue. Earlier studies have used TSPO targeting PET tracers in assessment of inflammatory burden in RA. Van der Laken et al. showed that there was higher expression of TSPO$^+$ cells measured with $^{[11}\text{C}]\text{PK11195}$ in RA patients with severe synovial edema compared to patients with mild edema. Narayan et al. used autoradiography and PBR-28 PET ligand to show an elevated level of fibroblast-like synoviocytes and activated macrophages in the synovium of RA patients over healthy control. This is the first study to examine the use of $^{[18}\text{F}]\text{FEPPA}$ PET in the assessment of OA specifically. $^{[18}\text{F}]\text{FEPPA}$ has advantages over earlier generation TSPO targeting radioligands in binding affinity and lipophilicity. $^{[11}\text{C}]\text{PK11195}$ is limited by high background signal and low SNR.

The synovial intima consists of mainly of macrophages and fibroblast-like synoviocytes. Infiltration of immune cells occurs during chronic synovitis. This study cohort had synovial tissue with between 46.1 – 100% CD68$^+$ cells. This suggests that a large proportion of synovial intima tissue consisted of CD68$^+$ cells which is a widely accepted marker for macrophages. All participants had highly activated macrophages with a mean value of $90.9\% \pm 11.4\%$ of all CD68$^+$ cells in the synovial intima, also overexpressing TSPO.
Validation of in vivo PET/MRI scans to accurately quantify activated macrophage content in knee synovial tissue showed a correlation between $[^{18}\text{F}]$FEPPA PET SUV$_{\text{mean}}$ and IF TSPO immunofluorescent signal that was not significant. However, a similar trend in Spearman r value was observed as in autoradiographic validation ($r = 0.90$, $p = 0.0833$). In vivo imaging will be needed to understand the relationship between inflammation and OA disease progression. Previous studies have shown the ability to track macrophages in vivo through other nuclear medicine approaches$^{116,117}$ or MRI$^{118,119}$. In addition, similar studies have looked at assessing macrophage activation in RA using older generation TSPO targeting tracers or assess features of OA using sodium fluoride or fluorodeoxyglucose PET tracers$^{89,92,120}$. These results are promising and represent a novel progression in assessment of synovitis by using a next generation PET tracer ($[^{18}\text{F}]$FEPPA) to target synovial macrophages in combination with excellent soft tissue contrast with 3T MRI sequences. This imaging protocol may allow for assessment of the relationship between macrophages and disease progression, validate future anti-inflammatory disease interventions, or investigate the role of macrophages in mediating the activation and proliferation of fibroblasts in the development of fibrosis in OA. However, more participants may need to be imaged to validate the in vivo PET/MRI scan protocol$^{119}$.

The relationship between chronic synovitis and fibrosis in OA is not clear. Synovial fibrosis is a characteristic of synovitis that can occur during the progression of OA. Fibrosis is characterized by excessive fibroblast proliferation and an imbalance between collagen synthesis and break-down. Studies have supported that inflammation is observed more in early stages of OA and fibrosis reported later in OA progression$^{121,122}$. It is possible that the regulatory function of macrophages to control fibroblast differentiation and fibrin deposition may be lost over time as OA progresses, due to chronic activation. This may lead to a gradual loss of fibroblast regulation over time and a slow imbalance towards fibrosis in the synovial tissue. It may be useful to study macrophage activation longitudinally to assess the relationship between chronic macrophage activation, fibroblast activity, and development of knee stiffness in OA.
2.5 Conclusion

\[^{18}\text{F}]\text{FEPPA}\] uptake in end-stage OA knee synovial tissue was validated to correlate to the true macrophage activation of that tissue. A Clinical \[^{18}\text{F}]\text{FEPPA}\] PET/MRI protocol showed similar trends in correlation, but more samples will be needed to draw definitive conclusions. \[^{18}\text{F}]\text{FEPPA}\] PET may be a useful tool to assess the relationship between macrophage activation and the progression of osteoarthritis.
Chapter 3

3 Conclusions and Future Directions

3.1 Overview of Objectives

Osteoarthritis is the most common joint affecting disease and is increasing in prevalence because of an aging demographic and rising rates of obesity\textsuperscript{123,124}. The cost of treatment for this disease is relatively high when compared to other musculoskeletal disorders because even during early stages patients consume health care resources at almost double the rate of the general population\textsuperscript{125}. The projected economic burden on the Canadian healthcare system is $7.6 billion by 2031, an increase of 160\% over 20 years\textsuperscript{126}. The knee joint is a complex structure that relies on the coordination and communication of many tissues to maintain healthy homeostasis. An understanding of the inflammatory process may be critical to successfully modify the course of this debilitating disease\textsuperscript{13}. The synovium regulates joint homeostasis and macrophages within the synovium are the dominant immune cell that secrete inflammatory cytokines to regulate inflammation\textsuperscript{16,63}. Imaging active macrophages has been proposed through targeting overexpression of TSPO with PET radioligands\textsuperscript{127}. \textsuperscript{18}F\textsuperscript{18}F\textsuperscript{18}F\textsuperscript{FEPPA}\textsuperscript{FEPPA} is a 2\textsuperscript{nd} generation PET tracer that can target activated macrophages\textsuperscript{128}. The objective of this thesis work was to examine the use of hybrid \textsuperscript{18}F\textsuperscript{FEPPA} PET/MRI for the accurate quantification of macrophage activation in a non-invasive, clinical scan.

3.2 Summary of Results

In chapter 2 of this thesis, \textsuperscript{18}F\textsuperscript{FEPPA} uptake in knee synovial tissue was assessed using autoradiography and strongly correlated to the actual TSPO content of that tissue measured through TSPO immunofluorescence (r = 0.85, p = 0.0029). This suggests that \textsuperscript{18}F\textsuperscript{FEPPA} may be useful in the assessment of knee synovitis in patients with end-stage OA. This is the first study to assess the use \textsuperscript{18}F\textsuperscript{FEPPA} in knee tissues in the context of OA. Previous studies have looked at imaging synovitis in RA or used other PET tracers...
to measure features of OA\textsuperscript{89,114}. The next part of chapter 2 began to explore the use of a clinical \textsuperscript{18}F\textsuperscript{FEPPA} PET/MRI scan to assess macrophage activation \textit{in vivo}. 6 participants were imaged and SUV\textsubscript{mean} in the synovium was calculated. TSPO IF from adjacent tissue sections correlated strongly to \textsuperscript{18}F\textsuperscript{FEPPA} tracer SUV\textsubscript{mean} (r = 0.9, p = 0.083). This suggests that there is also a strong correlation between \textsuperscript{18}F\textsuperscript{FEPPA} PET/MRI and the true macrophage activity of knee synovial tissue, however, more samples would be needed to draw definitive conclusions.

Next, chapter 2 explored the relationship between \textsuperscript{18}F\textsuperscript{FEPPA} uptake in knee synovial tissue and histological grading for important OA factors. There was no significant correlation between \textsuperscript{18}F\textsuperscript{FEPPA} uptake and any of the histological OA grading. Studies have shown an association with early OA synovitis and late-stage OA fibrosis\textsuperscript{63,129}. There was no correlation observed between fibrosis and macrophage activity in chapter 2. This could be because there is a more complicated relationship between macrophage regulation of fibroblasts or simply because the exact time in the trajectory of each patient’s OA pathology was unknown.

### 3.3 Future Directions

This thesis validated the use of \textsuperscript{18}F\textsuperscript{FEPPA} PET/MRI as a potential imaging modality to assess inflammation in knee synovial tissue. \textsuperscript{18}F\textsuperscript{FEPPA} imaging is costly and exposes the patient to ionizing radiation, so the goal of this thesis was to validate an imaging protocol and provide insight on appropriate imaging parameters, image processing, as well as assisting in sample size calculations before more resources are invested in future studies.

Future work will build on existing data and sample size estimates have been calculated based on the Spearman correlations from the thesis work, a probability to reject the null hypothesis (\(\alpha = 0.05\)), and a probability to reject the null hypothesis under the alternative hypothesis (\(\beta = 0.20\))(Table 6). Validation of the PET/MRI protocol will only require 7 participants (2 more in addition to existing work) assuming the correlation remains
consistent. Correlation between PET SUV and PROMs were highly variable and reaching the power required for significance would require very large sample sizes. When comparing only correlation strengths greater than \( r = 0.50 \) the estimated population size is much more reasonable. A sample size of 25 participants (20 additional) will likely be enough to validate many of the strong associations between macrophage activation measured in PET/MRI scans and PROMs (Table 6).

**Table 6: Expected sample size calculations between clinical PET [\(^{18}\)F]FEPPA SUV, immunofluorescence, and PROMs.**

<table>
<thead>
<tr>
<th>Correlation to PET SUV</th>
<th>Spearman r</th>
<th>Expected Sample Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSPO immunofluorescence</td>
<td>0.90</td>
<td>7</td>
</tr>
<tr>
<td>UCLA</td>
<td>0.12</td>
<td>543</td>
</tr>
<tr>
<td>VR-12 (PCS)</td>
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<td>10</td>
</tr>
<tr>
<td>VR-12 (MCS)</td>
<td>0.20</td>
<td>194</td>
</tr>
<tr>
<td>EQ-5D-5L</td>
<td>0.09</td>
<td>967</td>
</tr>
<tr>
<td>WOMAC (Pain)</td>
<td>-0.64</td>
<td>17</td>
</tr>
<tr>
<td>WOMAC (Stiffness)</td>
<td>-0.75</td>
<td>11</td>
</tr>
<tr>
<td>WOMAC (Function)</td>
<td>-0.17</td>
<td>269</td>
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<tr>
<td>OKS</td>
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<td>19</td>
</tr>
<tr>
<td>KSS</td>
<td>-0.54</td>
<td>25</td>
</tr>
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</table>

Future in house research has already begun to use this imaging protocol to characterize metal and plastic wear debris in patients needing hip revision implant surgery. Also, growing evidence supports the idea that gut microbiota diversity may modulate the immune system and have a significant impact of both RA and OA pathology\(^{130,131}\). Future
work will explore whether patient’s gut health measured by microbiota diversity is correlated to heightened immune response to hip implant wear debris.

Disease modifying treatments are currently being researched that target many pro-inflammatory mediators in the joint microenvironment\textsuperscript{132,133}. The imaging protocol developed in this thesis could be useful to explore why anti-inflammatory injections and therapies have been unsuccessful in modifying the course of OA. Future treatments could also be validated for effectiveness in long-term, longitudinal studies without the need for invasive tissue sampling.

### 3.4 Conclusions

This thesis set out to evaluate the potential of \textsuperscript{18}FFEPPA PET/MRI in accurately quantifying synovitis in patients with end-stage OA. The results supported that \textsuperscript{18}FFEPPA PET/MRI was valid in quantifying macrophage activation and suggests that this imaging protocol may be a useful tool to probe the relationship between inflammation and other important OA features like pain, stiffness, and loss of function. OA is a complex disease that involves dysregulation of many joint tissues and validated imaging of inflammation may be an important facet of an OA treatment solution.
References


67. Remst DFG, Blaney Davidson EN, van der Kraan PM. Unravelling osteoarthritis-related synovial fibrosis: a step closer to solving joint


   http://useast.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=2:43162420-43163420;v=rs6971;vdb=variation;vf=184151621


110. Moriya S, Miki Y, Matsuno Y, Okada M. Three-dimensional double-echo steady-state (3D-DESS) magnetic resonance imaging of the knee:


Appendices

Appendix A: Health Canada Authorization to use a Radiopharmaceutical in a Basic Research Study

Health Canada
Santé Canada

Biologic and Radiopharmaceutical Drugs Directorate
100 Elginette Drive
LCDC Building,
Tunney’s Pasture, A.L. 0501C
Ottawa, Ontario
K1A 0K9

September 2, 2021

AUTHORIZATION TO SELL

Dear Dr. Lusting:

The Basic Research Application - PER for 18F-FEPPA Injection, submitted on August 18, 2021, concerning Protocol # Fibrous TKA entitled: "Quantification of intra-articular fibrosis in patients before total knee arthroplasty and implant revision using metal reduction MEI and 18FF-EPPA PET tracer", was received.

In accordance with Part C, Division 3 of the Food and Drug Regulations, you are authorized to sell or import the study drug for the purpose of a basic research study.

Please note that for each new Canadian site to be included in this trial, you must submit a completed Appendix 2 – Study Site Information and Research Ethics Board Authorization prior to initiating the trial at that site. A new or revised Appendix 2 should be emailed to...

You are reminded to submit reports for all serious and unexpected adverse drug reactions (ADR) directly to the Biologic and Radiopharmaceutical Drugs Directorate in accordance with Section C.09.314 of the Food and Drug Regulations.

Sincerely,

This document has been signed electronically using the Health Canada DocuBridge system.
Appendix B: Human Radionuclide Safety and Scientific Review Committee (HRSSRC) Letter of No Objection

Human Radionuclide Safety and Scientific Review Committee

LETTER OF NO OBJECTION

The Human Radionuclide Safety and Scientific Review Committee (the “Committee”) for London Health Sciences Centre and St. Joseph’s Health Care London has reviewed the scientific and radiation safety aspects of the research project listed below.

The Committee has no objections to the study commencing, provided all necessary authorizations are in place. The study must be executed only as specified by the approved protocol. Any deviations must be reported within 24 hours to the Committee Chair (Chief/Chair Nuclear Medicine) and the Radiation Safety Officer overseeing the corresponding CNSC license.

This approval is time limited, and expires on the date listed below. The Committee will consider extensions upon request.

<table>
<thead>
<tr>
<th>STUDY NAME</th>
<th>Quantification of intra-articular fibrosis in patients before total knee arthroplasty and implant revision using metal reduction MRI and [18F]PEPPA PET tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRINCIPAL INVESTIGATOR</td>
<td>Brent Lanting</td>
</tr>
<tr>
<td>PROTOCOL VERSION</td>
<td>Protocol updated. Consent V10, dated March 10 2021</td>
</tr>
<tr>
<td>CNSC LICENCE AND RSO</td>
<td>Human Research Studies 13183-12-24 X Ben Reyes</td>
</tr>
<tr>
<td>RESEARCH LOCATION</td>
<td>St Joseph’s / Grosvenor</td>
</tr>
<tr>
<td>EXPIRY DATE</td>
<td>2022 July 01</td>
</tr>
</tbody>
</table>
Appendix C: Approval notice to begin study activity.

LAWSON FINAL APPROVAL NOTICE

LAWSON APPROVAL NUMBER: R-21-506

PROJECT TITLE: Quantification of intra-articular fibrosis in patients before total knee arthroplasty and implant revision using metal reduction MRI and [18F]FEPPA PET tracer.

PRINCIPAL INVESTIGATOR: Dr. Brent Lanting

LAWSON APPROVAL DATE: 12/10/2021

ReDA ID: 10914

Overall Study Status: Active

Please be advised that the above project was reviewed by Lawson Administration and the project was approved.

“COVID-19: Please note that Lawson is continuing to review and approve research studies. However, this does not mean the study can be implemented during the COVID-19 pandemic. Principal investigators, in consultation with their program leader or Chair/Chief, should use their judgment and consult Lawson’s research directive and guidelines to determine the appropriateness of starting the study. Compliance with hospital, Lawson, and government public health directives and participant and research team safety supersedes Lawson Approval.”

Please provide your Lawson Approval Number (R#) to the appropriate contact(s) in supporting departments (eg. Lab Services, Diagnostic Imaging, etc.) to inform them that your study is starting. The Lawson Approval Number must be provided each time services are requested.

Dr. David Hill
V.P. Research
Lawson Health Research Institute
Appendix D: Univeristy of California, Los Angeles (UCLA) Activity Score

Name: __________
Date: __________

Hip and Knee Screening Assessment

1. What part of your body are you visiting the clinic about today? (select all that apply)

<table>
<thead>
<tr>
<th>Right Hip</th>
<th>Left Hip</th>
<th>Right Knee</th>
<th>Left Knee</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

2. What is your overall satisfaction with your replacement surgery?

<table>
<thead>
<tr>
<th>Very Satisfied</th>
<th>Satisfied</th>
<th>Neutral</th>
<th>Dissatisfied</th>
<th>Very Dissatisfied</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Hip</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Left Hip</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Right Knee</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Left Knee</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

UCLA Activity Score

Check one box that best describes current activity level.

- 10: Regularly participates in impact sports such as jogging, tennis, skiing, aeroabatics, ballet, heavy labor or backpacking
- 9: Sometimes participates in impact sports
- 8: Regularly participates in very active events, such as golf or bowling
- 7: Regularly participates in active events such as bicycling
- 6: Regularly participates in moderate activities such as swimming or could do unlimited housework or shopping
- 5: Sometimes participates in moderate activities
- 4: Regularly participates in mild activities such as walking, limited housework and limited shopping
- 3: Sometimes participates in mild activities
- 2: Mostly Inactive or restricted to minimum activities of daily living
- 1: Wholly Inactive, dependent on others, cannot leave residence
Appendix E: Veterans RAND 12 (VR-12)

VR-12 SURVEY
The next set of questions asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

1. In general, would you say your health is:

<table>
<thead>
<tr>
<th>Excellent</th>
<th>Very Good</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

2. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

<table>
<thead>
<tr>
<th>a. Moderate activities such as moving a table, pushing a vacuum cleaner, bowling or playing golf</th>
<th>Yes, Limited A Lot</th>
<th>Yes, Limited A Little</th>
<th>No, Not Limited At All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| b. Climbing several flights of stairs             | 0                 | 0                    | 0                     |

3. During the past 4 weeks, how much time have you had any of the following health problems with your work or other regular daily activities as a result of your physical health?

<table>
<thead>
<tr>
<th>a. Accomplished less than you would like</th>
<th>No, None of the time</th>
<th>Yes, A little of the time</th>
<th>Yes, Some of the time</th>
<th>Yes, Most of the time</th>
<th>Yes, All of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| b. Were limited in the kind of work or other activities. | 0 | 0 | 0 | 0 | 0 |

4. During the past 4 weeks, how much time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

<table>
<thead>
<tr>
<th>a. Accomplished less than you would like</th>
<th>No, None of the time</th>
<th>Yes, A little of the time</th>
<th>Yes, Some of the time</th>
<th>Yes, Most of the time</th>
<th>Yes, All of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| b. Didn't do work or other activities as carefully as usual. | 0 | 0 | 0 | 0 | 0 |

5. During the past 4 weeks, how much did pain interfere with your normal work (including both work and outside the home and housework)?

<table>
<thead>
<tr>
<th>Not at All</th>
<th>A little bit</th>
<th>Moderately</th>
<th>Quite a bit</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

<table>
<thead>
<tr>
<th>6. How much of the time during the past 4 weeks</th>
<th>All of the time</th>
<th>Most of the time</th>
<th>A Good bit of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Have you felt calm and peaceful?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>b. Did you have a lot of energy?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>c. Have you felt downhearted and depressed?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

7. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

<table>
<thead>
<tr>
<th></th>
<th>All of the time</th>
<th>Most of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

Now, we'd like to ask you some questions about how your health may have changed.

<table>
<thead>
<tr>
<th>Compared to one year ago</th>
<th>Much better</th>
<th>Slightly better</th>
<th>About the same</th>
<th>Slightly worse</th>
<th>Much worse</th>
</tr>
</thead>
<tbody>
<tr>
<td>8. How would you rate your physical health in general now?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>9. How would you rate your emotional problems (such as feeling anxious, depressed or irritable) now?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>
Appendix F: European Quality of Life – 5 Dimension – 5 Levels (EQ-5D-5L)

Under each heading, please tick the ONE box that best describes your health TODAY

**MOBILITY**
- I have no problems in walking about
- I have slight problems in walking about
- I have moderate problems in walking about
- I have severe problems in walking about
- I am unable to walk about

**SELF-CARE**
- I have no problems washing or dressing myself
- I have slight problems washing or dressing myself
- I have moderate problems washing or dressing myself
- I have severe problems washing or dressing myself
- I am unable to wash or dress myself

**USUAL ACTIVITIES (e.g. work, study, housework, family or leisure activities)**
- I have no problems doing my usual activities
- I have slight problems doing my usual activities
- I have moderate problems doing my usual activities
- I have severe problems doing my usual activities
- I am unable to do my usual activities

**PAIN / DISCOMFORT**
- I have no pain or discomfort
- I have slight pain or discomfort
- I have moderate pain or discomfort
- I have severe pain or discomfort
- I have extreme pain or discomfort

**ANXIETY / DEPRESSION**
- I am not anxious or depressed
- I am slightly anxious or depressed
- I am moderately anxious or depressed
- I am severely anxious or depressed
- I am extremely anxious or depressed
- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = 

The best health you can imagine

100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0

The worst health you can imagine

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Appendix G: Western Ontario and McMaster University Osteoarthritis Index (WOMAC)

WOMAC SURVEY

In Sections A, B and C, questions will be asked about your hip and/or knee replacement surgery. You should give your answers by circling the number for the corresponding question.

A. Think about the pain you felt in your hip/knee during the last 48 hours

<table>
<thead>
<tr>
<th>Question: How much pain do you have?</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Walking on a flat surface</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2. Going up or down stairs</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3. At night while in bed, pain disturbs your sleep</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4. Sitting or lying</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5. Standing upright</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

B. Think about the stiffness (not pain) you felt in your hip/knee during the last 48 hours. Stiffness is a sensation of decreased ease in moving your joint

<table>
<thead>
<tr>
<th>Question: How severe is your stiffness after first awakening in the morning?</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. How severe is your stiffness after first awakening in the morning?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Question: How severe is your stiffness after sitting, lying or resting later in the day?</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. How severe is your stiffness after sitting, lying or resting later in the day?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

C. Think about the difficulty you had in doing the following daily physical activities due to your hip/knee during the last 48 hours. By this we mean your ability to move around and look after yourself.

<table>
<thead>
<tr>
<th>Question: What degree of difficulty do you have?</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Descending Stairs</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2. Ascending Stairs</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3. Rising from sitting</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4. Standing</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5. Bending to the floor</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6. Walking on a flat surface</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7. Getting in and out of car, or on off a bus</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8. Going shopping</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9. Putting on your socks or stockings</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10. Rising from bed</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>11. Taking off your socks or stockings</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>12. Lying in bed</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>13. Getting in or out of the bath</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>14. Sitting</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>15. Getting on or off the toilet</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>16. Performing heavy domestic duties</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>17. Performing light domestic duties</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
Appendix H: Oxford Knee Score

**OXFORD KNEE SCORE**

Name: \\
Date: \\

PROBLEMS WITH YOUR KNEE

Check (O) one box for every question. Please respond for both left and right if both are replaced.

<table>
<thead>
<tr>
<th>1. During the past 4 weeks.....</th>
<th>How would you describe the pain you usually have from your knee?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>LEFT</td>
<td>O</td>
</tr>
<tr>
<td>RIGHT</td>
<td>O</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. During the past 4 weeks.....</th>
<th>Have you had any trouble with washing and drying yourself (all over) because of your knee?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No trouble at all</td>
</tr>
<tr>
<td>LEFT</td>
<td>O</td>
</tr>
<tr>
<td>RIGHT</td>
<td>O</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. During the past 4 weeks.....</th>
<th>Have you had any trouble getting in and out of a car or using public transportation because of your knee? (whichever you tend to use)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No trouble at all</td>
</tr>
<tr>
<td>LEFT</td>
<td>O</td>
</tr>
<tr>
<td>RIGHT</td>
<td>O</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. During the past 4 weeks.....</th>
<th>How long have you been able to walk before pain from your knee becomes severe? (with or without a stick/cane)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No pain/More than 30 minutes</td>
</tr>
<tr>
<td>LEFT</td>
<td>O</td>
</tr>
<tr>
<td>RIGHT</td>
<td>O</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5. During the past 4 weeks.....</th>
<th>After a meal (sitting at a table), how painful has it been for you to stand up from a chair because of your knee?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not at all painful</td>
</tr>
<tr>
<td>LEFT</td>
<td>O</td>
</tr>
<tr>
<td>RIGHT</td>
<td>O</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6. During the past 4 weeks.....</th>
<th>Have you been limping when walking because of your knee?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rarely/never</td>
</tr>
<tr>
<td>LEFT</td>
<td>O</td>
</tr>
<tr>
<td>RIGHT</td>
<td>O</td>
</tr>
</tbody>
</table>
7. **During the past 4 weeks....**

**Could** you kneel down and get up again afterwards?

<table>
<thead>
<tr>
<th>Yes, easily</th>
<th>With little difficulty</th>
<th>With moderate difficulty</th>
<th>With extreme difficulty</th>
<th>No, impossible</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEFT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>RIGHT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

8. **During the past 4 weeks....**

Have you been troubled by pain from your knee in bed at night?

<table>
<thead>
<tr>
<th>No nights</th>
<th>Only 1 or 2 nights</th>
<th>Some nights</th>
<th>Most nights</th>
<th>Every night</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEFT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>RIGHT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

9. **During the past 4 weeks....**

How much has pain from your knee interfered with your usual work (including housework)?

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little bit</th>
<th>Moderately</th>
<th>Greatly</th>
<th>Totally</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEFT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>RIGHT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

10. **During the past 4 weeks....**

Have you felt that your knee might suddenly "give out" or let you down?

<table>
<thead>
<tr>
<th>Rarely/never</th>
<th>Sometimes, or just at first</th>
<th>Often, not just at first</th>
<th>Most of the time</th>
<th>All of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEFT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>RIGHT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

11. **During the past 4 weeks....**

**Could** you do the household/grocery shopping on your own?

<table>
<thead>
<tr>
<th>Yes, easily</th>
<th>With little difficulty</th>
<th>With moderate difficulty</th>
<th>With extreme difficulty</th>
<th>No, impossible</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEFT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>RIGHT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

12. **During the past 4 weeks....**

**Could** you walk down one flight of stairs?

<table>
<thead>
<tr>
<th>Yes, easily</th>
<th>With little difficulty</th>
<th>With moderate difficulty</th>
<th>With extreme difficulty</th>
<th>No, impossible</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEFT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>RIGHT</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Finally, please make sure that you have answered each question.  
Thank you very much.
## Appendix I: Knee Society Score - Patient Portion

### Symptoms

**1. Pain with level walking**

<table>
<thead>
<tr>
<th>Score</th>
<th>None</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Patient Satisfaction

1. **Currently, how satisfied are you with the pain level of your knee while sitting?**

<table>
<thead>
<tr>
<th>Level</th>
<th>(8 points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Satisfied (8 pts)</td>
<td>Satisfied (6 pts)</td>
</tr>
</tbody>
</table>

2. **Currently, how satisfied are you with the pain level of your knee while lying in bed?**

<table>
<thead>
<tr>
<th>Level</th>
<th>(8 points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Satisfied (8 pts)</td>
<td>Satisfied (6 pts)</td>
</tr>
</tbody>
</table>

3. **Currently, how satisfied are you with your knee function while getting out of bed?**

<table>
<thead>
<tr>
<th>Level</th>
<th>(8 points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Satisfied (8 pts)</td>
<td>Satisfied (6 pts)</td>
</tr>
</tbody>
</table>

4. **Currently, how satisfied are you with your knee function while performing light household duties?**

<table>
<thead>
<tr>
<th>Level</th>
<th>(8 points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Satisfied (8 pts)</td>
<td>Satisfied (6 pts)</td>
</tr>
</tbody>
</table>

5. **Currently, how satisfied are you with your knee function while performing leisure recreational activities?**

<table>
<thead>
<tr>
<th>Level</th>
<th>(8 points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Satisfied (8 pts)</td>
<td>Satisfied (6 pts)</td>
</tr>
</tbody>
</table>

Maximum total points (40 points)
# PATIENT EXPECTATION

(To be completed by patient)

Compared to what you expected before your knee replacement:

## 1- My expectations for pain relief were...

- **Too High**: "I'm a lot worse than I thought" (1 pt)
- **Too High**: "I'm somewhat worse than I thought" (2 pts)
- **Just Right**: "My expectations were met" (3 pts)
- **Too Low**: "I'm somewhat better than I thought" (4 pts)
- **Too Low**: "I'm a lot better than I thought" (5 pts)

## 2- My expectations for being able to do my normal activities of daily living were...

- **Too High**: "I'm a lot worse than I thought" (1 pt)
- **Too High**: "I'm somewhat worse than I thought" (2 pts)
- **Just Right**: "My expectations were met" (3 pts)
- **Too Low**: "I'm somewhat better than I thought" (4 pts)
- **Too Low**: "I'm a lot better than I thought" (5 pts)

## 3- My expectations for being able to do my leisure, recreational or sports activities were...

- **Too High**: "I'm a lot worse than I thought" (1 pt)
- **Too High**: "I'm somewhat worse than I thought" (2 pts)
- **Just Right**: "My expectations were met" (3 pts)
- **Too Low**: "I'm somewhat better than I thought" (4 pts)
- **Too Low**: "I'm a lot better than I thought" (5 pts)

**Maximum total points (15 points)**

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### FUNCTIONAL ACTIVITIES

(To be completed by patient)

#### WALKING AND STANDING (30 points)

1. Can you walk without any aids (such as a cane, crutches or wheelchair)?
   - Yes
   - No
   - (0 points)

2. If no, which of the following aid(s) do you use?
   - wheelchair (-10 pts)
   - walker (-8 pts)
   - crutches (-8 pts)
   - two canes (-6 pts)
   - one crutch (-4 pts)
   - one cane (-4 pts)
   - knee sleeve/brace (2 pts)
   - other

   (-10 points)

3. Do you use these aid(s) because of your knees?
   - Yes
   - No
   - (0 points)

4. For how long can you stand (with or without aid) before sitting due to knee discomfort?
   - cannot stand (0 pts)
   - 0-5 minutes (3 pts)
   - 6-15 minutes (6 pts)
   - 16-30 minutes (9 pts)
   - 31-50 minutes (12 pts)
   - more than an hour (15 pts)

   (15 points)

5. For how long can you walk (with or without aid) before stopping due to knee discomfort?
   - cannot walk (0 pts)
   - 0-5 minutes (3 pts)
   - 6-15 minutes (6 pts)
   - 16-30 minutes (9 pts)
   - 31-50 minutes (12 pts)
   - more than an hour (15 pts)

   (15 points)

**Maximum points (30 points)**

---

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### STANDARD ACTIVITIES (30 points)

<table>
<thead>
<tr>
<th>Activity</th>
<th>No Bother</th>
<th>Slight</th>
<th>Moderate</th>
<th>Severe</th>
<th>Very Severe</th>
<th>Cannot Do (Because of Knee)</th>
<th>I Never Do This</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Walking on an uneven surface</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - Turning or pivoting on your leg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - Climbing up or down a flight of stairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 - Getting up from a low couch or a chair without arms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - Getting into or out of a car</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - Moving laterally (stepping to the side)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Maximum points (30 points)  

### ADVANCED ACTIVITIES (25 points)

<table>
<thead>
<tr>
<th>Activity</th>
<th>No Bother</th>
<th>Slight</th>
<th>Moderate</th>
<th>Severe</th>
<th>Very Severe</th>
<th>Cannot Do (Because of Knee)</th>
<th>I Never Do This</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Climbing a ladder or step stool</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - Carrying a shopping bag for a block</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - Squatting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 - Kneeling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - Running</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Maximum points (25 points)
### DISCRETIONARY KNEE ACTIVITIES (15 points)

Please check 3 of the activities below that you consider *most important* to you.  
(Please do not write in additional activities)

- **Recreational Activities**
  - Swimming
  - Golfing (18 holes)
  - Road Cycling (>30 mins)
  - Gardening
  - Bowling
  - Racquet Sports (Tennis, Racquetball, etc.)
  - Distance Walking
  - Dancing / Ballet
  - Stretching Exercises (stretching out your muscles)

- **Workout and Gym Activities**
  - Weight-lifting
  - Leg Extensions
  - Stair-Climber
  - Stationary Biking / Spinning
  - Leg Press
  - Jogging
  - Elliptical Trainer
  - Aerobic Exercises

Please copy all 3 checked activities into the empty boxes below.

### How much does your knee bother you during each of these activities?

<table>
<thead>
<tr>
<th>Activity (Please write the 3 activities from list above)</th>
<th>no bother</th>
<th>slight</th>
<th>moderate</th>
<th>severe</th>
<th>very severe</th>
<th>cannot do (because of knee)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

1. 

2. 

3. 

Maximum points (15 points) 

Maximum total points (100 points) 

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Appendix J: Knee Society Score - Surgeon Portion

**OBJECTIVE KNEE INDICATORS**  
(To be completed by surgeon)

### ALIGNMENT
1. Alignment: measured on AP standing Xray (Anatomic Alignment)  
   - Neutral: 2-10 degrees valgus (25 pts)
   - Varus: < 2 degrees valgus (-10 pts)
   - Valgus: > 10 degrees valgus (-10 pts)

### INSTABILITY
2. Medial / Lateral Instability: measured in full extension  
   - None (15 pts)
   - Little or < 5 mm (10 pts)
   - Moderate or 5 mm (5 pts)
   - Severe or > 5 mm (0 pts)

3. Anterior / Posterior Instability: measured at 90 degrees  
   - None (10 pts)
   - Moderate < 5 mm (5 pts)
   - Severe > 5 mm (0 pts)

### JOINT MOTION
4. Range of motion (1 point for each 5 degrees)

<table>
<thead>
<tr>
<th>Deformities</th>
<th>Minus Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion Contracture</td>
<td></td>
</tr>
<tr>
<td>1-5 degrees</td>
<td>(-2 pts)</td>
</tr>
<tr>
<td>6-10 degrees</td>
<td>(-5 pts)</td>
</tr>
<tr>
<td>11-15 degrees</td>
<td>(-10 pts)</td>
</tr>
<tr>
<td>&gt; 15 degrees</td>
<td>(-15 pts)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extension Lag</th>
<th>Minus Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 degrees</td>
<td>(-5 pts)</td>
</tr>
<tr>
<td>10-20 degrees</td>
<td>(-10 pts)</td>
</tr>
<tr>
<td>&gt; 20 degrees</td>
<td>(-15 pts)</td>
</tr>
</tbody>
</table>
Appendix K: Letter of Information and Consent

LETTER OF INFORMATION AND CONSENT

Title of Study: QUANTIFICATION OF INTRA-ARTICULAR FIBROSIS IN PATIENTS BEFORE TOTAL KNEE ARTHROPLASTY AS AFTER IMPLANT REVISION USING METAL REDUCTION MRI AND [18F]FDG PET TRACER.

Document Title: Letter of Information and Consent

Principal Investigator: Dr. Brent Lanting, MD MSc FRSC

Co-Investigators: Dr. Matthew Teeter, PhD
Dr. Thomas Appleton, MD, PhD, FRCPC
Dr. Stephany Pritchett, MD
Dr. Vishal Kalia, MD, FRCR
Dr. Jonathan Thiessen, PhD
Dr. Steven MacDonald, MD RCPSC

Research Staff:

Sponsor/Funder Information:
Lawson Health Research Institute/Internal Research Fund (IRF).

Conflict of Interest:
There are no known conflicts of interest in this study.

Invitation to Participate:

You are being invited to voluntarily participate in a research study because you will be having knee surgery. We are looking at two groups of people: those having their first knee replacement with no knee stiffness and those having a revision knee replacement due to stiffness. This study will be conducted as part of the Master of Science (MSc) degree of a student on the research staff through Western University. The purpose of the study is to find a non-invasive way to determine knee inflammation around metal implants. This letter of information describes the research study and your role as a participant. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends, and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

Why is this study being done?
Total Knee replacement surgery can lead to inflammation, scarring, and stiffness in the joint over time. We want to study ways to decrease the stiffness that accumulates but the metal in joint implants make it difficult to take images of what is happening around the joint. We want to inject a tracer that can show inflammation in the body and scan your knee to see the location and amount of inflammation. Your surgeon will save a sample of knee tissue during your surgery that we can use to check the actual inflammation and fibrosis levels that were in your knee and then compare those results to what we found in our scars.

How long will you be in this study?

It is expected that you will be in this study for 2 to 4 months depending on the time between your presurgical appointment and the day of your surgery. There will be 2 visits with research staff and each visit will take approximately 1 hour.

What are the study procedures?

If you agree to participate you will be part of a group of 12 participants, those that are either undergoing their first knee replacement surgery or those that are having a revision knee replacement for knee stiffness. If you want to be a participant in this study, you will sign and return the consent form at the end of this letter after taking the time you need to understand what will happen in this study and have any questions answered. If you agree to participate a research staff member will meet you at your presurgical appointment at University Hospital (UH). We will perform a walking test to broadly check your knee function. We will time how long it takes for you to get up from sitting on a chair and walk forward 3 meters, turn around, and walk back to sit down in the chair. During your presurgical appointment you will have your blood taken for a standard workup. We will add an additional vial to be drawn for testing related to this study. This extra vial is to check to make sure you are compatible with the substance that is injected for the Positron Emission Tomography (PET) imaging technique we will be using. This substance is called [18F]FEPDA and is a PET tracer that is not currently approved by Health Canada for use in the way described in this research but Health Canada has authorized its use for this study. The first appointment will add about 10 minutes to your normal presurgical appointment.

The blood test results will take about 1 week to come back and if you are not excluded from the study by your blood test results a research staff member will call you to set up a second appointment. This appointment will last about 1.5 hours and will require you to come to St. Joseph's Health Care to be given an intravenous PET tracer and scan your knee with MRI.

At the time of your knee replacement the surgeon will collect tissue samples from your knee. This tissue is normally removed during knee replacement but instead of discarding this tissue we will save it for the purposes of this study. Once all participants have finished their surgery and data has been collected and analyzed you will be contacted to see if you would like a follow up visit to discuss the findings of the study.
What are the risks and harms of participating in this study?

The possible risks and harms to you include:

Privacy risks – There is always a risk for breach of confidentiality of your data. All data collected will have no direct identifiers attached to it. Physical papers will be locked in a secure location and electronic data will be on an encrypted secure hospital drive.

Blood Sampling – Blood drawn at your initial appointment will be done by a qualified hospital nurse however some possible risks when having your blood taken include dizziness, fainting, and bruising.

PET tracer - The main risk involved in this study is in the administration of a low-level radioactive substance called a tracer. This will be given intravenously at your second appointment at St. Joseph’s hospital and will allow the research team to scan your knee. The radiation levels you will receive are low (about the same as a standard chest Xray) and all Government and Hospital safety regulations will be followed.

Claustrophobia risk – You will be required to lay in the scanning tunnel of the Positron Emission Tomography/Magnetic Resonance Imaging (PET/MRI) scanner. The width of the tunnel you will be in is 60cm or approximately 2 feet. You will be required to remain in the tunnel for the length of the scan (about 30 minutes) and some people may experience claustrophobic feelings while inside. There will be a technician and research staff member monitoring you by two-way audio and video throughout the procedure and you may end the scan at any time if needed.

What are the benefits of participating in this study?

You may not directly benefit from this study, but information gathered may provide benefits to society as a whole including increasing the longevity and decreasing stiffness in future people undergoing knee replacement surgery or revision.

Can participants choose to leave the study?

Your decision to take part in this study is voluntary. You may refuse to participate, or you may withdraw from the study at any time. Your decision not to participate or to withdraw from the study will not affect your other medical care at this site. If you choose to withdraw from the study, data collected up to the time of withdrawal will continue to be used for the study.
However, no new information will be collected. If you would like to withdraw from the study, you will need to provide written or verbal communication to the study coordinator.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

How will your information be kept confidential?

Information gathered in this research study may be published or presented in public forums, however your name and other identifying information will not be used or revealed. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law.

We will collect your name, address, postal code, telephone number, sex, age, and hospital personal identification number (PIN). This data will be collected to allow the research team to screen you for study eligibility and to communicate with you throughout the duration of the study.

We will do our best to protect the information collected in this study. All participants will be assigned a participant ID that will be used on all disclosed or disseminated documents. The “master participant key” that ties each participant to their identifiable data will be stored in a locked secure space or institutional network in the hospital and will only be accessible by research staff. All documents other than the master key will contain only your participant ID to keep your data safe.

Study data will be kept for 25 years and even if you withdraw from the study, your information collected before withdrawal will be retained to comply with Health Canada regulations. You do not waive any legal rights by signing the consent form. Representatives of Western University’s Health Science Research Ethics Board may require access to your study-related records to monitor the conduct of the research.

Qualified representatives of the Lawson Quality Assurance Education Program may look at your medical/clinical study records at the site where these records are held, for quality assurance (to check that the information collected for the study is correct and follows proper laws and guidelines).

Are participants compensated to be in this study?

You will not be compensated for your participation in this research however you will be provided a parking pass for your appointment at St. Joseph’s Healthcare.

What are the rights of participants?
Your participation in this study is voluntary. You may decide not to be in this study. Even if you consent to participate you have the right to not answer individual questions or to withdraw from the study at any time. If you choose not to participate or to leave the study at any time it will have no effect on your medical care.

We will give you any new information that may affect your decision to stay in the study.

**Whom do participants contact for questions?**

If you have questions about this research study, please contact:

Dr. Brent Lanting
Zachary Koudys

If you have any questions about your rights as a research participant or the conduct of this study, you may contact the Patient Relations Office at LHSC.

This letter is yours to keep for future reference.
WRITTEN CONSENT

Title of Study: QUANTIFICATION OF INTRA-ARTICULAR FIBROSIS IN PATIENTS BEFORE TOTAL KNEE ARTHROPLASTY AS AFTER IMPLANT REVISION USING METAL REDUCTION MRI AND [18F]FP-EPPA PET TRACER.

Document Title: Letter of Information and Consent

Principal Investigator: Dr. Brent Lanting, MD MSc FRCSC

Co-Investigators: Dr. Matthew Teter, PhD
Dr. Thomas Appleton, MD, PhD, FRCPC
Dr. Stephanie Pritchett, MD
Dr. Vishal Kalia, MD, FRCR
Dr. Jonathan Thiessen, MD
Dr. Steven MacDonald, MD RCPC

Research Staff:

I have read the Letter of Information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

CONTACT FOR FUTURE STUDIES Please initial on the appropriate line below.:  

___ I agree to be contacted for future research studies  
___ I do NOT agree to be contacted for future research studies

________________________  ___________________________  __________________________
Print name of participant  Signature  Date (DD/MM/YYYY)

My signature means that I have explained the study to the participant named above. I have answered all questions.

________________________  ___________________________  __________________________
Print name of person  Signature  Date (DD/MM/YYYY)

Obtaining consent

Version 2.2 2022/09/19 INITIALS
Curriculum Vitae

Zachary Koudys

EDUCATION

September
2009 – 2015
Bachelor of Science: Biochemistry
School of Chemistry and Biochemistry, The University of Windsor, Windsor, ON, Canada

January
2021 – Present
Master of Science, Department of Medical Biophysics
Schulich School of Medicine and Dentistry, Western University, London, ON, Canada
- Thesis Title: Assessing Inflammation in the Pathology of Knee Osteoarthritis
- Supervisors: Dr. Matthew Teeter, Dr. Jonathan Thiessen
- Expected completion: June 2023

June
2021 – Present
Collaborative Program in Musculoskeletal Health Research
Bone and Joint Institute, Western University, London, ON, Canada
- Research group focused on integrated approaches for the management of bone and joint diseases.
- Certification awarded in addition to confirmation of graduate degree.

June
2021 – Present
Collaborative Program in Molecular Imaging
Schulich School of Medicine and Dentistry, Western University, London, ON, Canada
- Research group that fosters collaboration between graduate programs to provide advanced interdisciplinary training in molecular imaging techniques.
- Certification awarded in addition to confirmation of graduate degree.

RESEARCH EXPERIENCE

September
2019 – 2021
Research Assistant
London Regional Cancer Program, London, ON, Canada
- Supervisor: Dr. Stephen Sawchuk
- Responsible for data collection, analysis, and manuscript preparation

**June 2021 – Present**

**Trainee Research Assistant**

Robarts Research Institute, London, ON, Canada

- Supervisor: Dr. Matthew Teeter
- Funding: Lawson Strategic Research Fund
- Project Title: PET/MRI assessment of the inflammatory response to wear debris after total hip arthroplasty.
- Assisted with ethics and regulatory approval. Consulted with other research team members on study logistics and procedures.

**January 2021 – Present**

**Master of Science Thesis Project**

University Hospital, Robarts Research Institute, and St. Joseph Health Care, London, ON, Canada

- Supervisors: Dr. Matthew Teeter, Dr. Jonathan Thiessen
- Funding: Lawson Internal Research Fund
- Project Title: Quantification of Macrophage Activity in Knee Synovial Tissue Using [18F]FEPPA PET Radioligand
- Responsible for all ethics and regulatory approval, patient screening and recruitment, data collection, surgical tissue transfer, data processing and analysis, and manuscript preparation.

**PUBLICATIONS**


**PRESENTATIONS (Peer Reviewed)**

1. Koudys ZJ, Lanting BA, Appleton CT, Thiessen JD, Teeter MG. [18F]FEPPA Autoradiography As a Measure of Macrophage Content in Knee Synovial Tissue.


**PRESENTATIONS (Not Peer Reviewed)**

