SRTP - Project Description Form #225

PART I:

Name of Schulich faculty member who will supervise the project	Tom Appleton
Supervisor's Schulich, Western, Hospital or Lawson Email	tom.appleton@sjhc.london.on.ca
Schulich Department	Medicine

PART II - Project Description

Title of Project	Joint-regenerative mechanisms in knee osteoarthritis

Background

Knee osteoarthritis (OA) causes pain and joint damage. OA has been described as a chronic (non-healing) wound, suggesting that endogenous regenerative (disease compensation) mechanisms fail to restore joint homeostasis in patients with progressive disease. We propose that enhancing and/or disinhibiting disease compensation mechanisms can reduce pain, inflammation, tissue damage, and progression to joint organ failure. However, it is difficult to differentiate joint healing mechanisms from pathophysiologic processes in studies comparing OA vs healthy controls, or between OA subgroups (e.g., early- vs late-stage), due to inherent variability among patients and the clinical heterogeneity of OA. In this exploratory study, we will overcome this methodologic challenge by comparing joints that are differentially affected by OA within the same patient.

Although knee OA causes debilitating symptoms, up to 70% of patients with symptomatic knee OA never require joint replacement for end-stage (decompensated) joint failure. Many patients experience partial (partially compensated) or complete (well compensated) resolution of joint symptoms for several years without progression of joint damage. These key clinical observations suggest that endogenous joint-healing mechanisms are sufficient to control OA for some patients, but insufficient for others.

Patients undergoing knee arthroplasty frequently have partially or well compensated OA in their contralateral knee. This clinical setting provides an ideal model with higher power to identify biological mechanisms of disease compensation, while controlling for numerous known and unknown patient-specific sources of variation. To identify joint regenerative mechanisms in this study, we will compare contralateral knees with OA that are i) partially, or ii) well compensated, vs the severely decompensated (surgical) knee. Since the joint lining (synovial tissue) supplies essential blood, oxygen, and nutrients to maintain joint homeostasis, and synovial inflammation (synovitis) is strongly associated with pain and joint damage in OA, we will identify joint-regenerative mechanisms in synovial tissues collected during arthroplasty.

Hypothesis

We hypothesize that contrasting partially or well compensated OA with decompensated OA between knees from the same patient will permit the identification of regenerative and pathophysiologic processes driving OA status. Further, more compensated OA will be associated with increased microvascularization and reduced perivascular edema, lining hyperplasia, and inflammatory cell infiltration, and with increased expression of anabolic and regenerative gene expression and pathways in synovial tissues.

Proposed Methodology

We will recruit an equal proportion of male and female participants (n=32) between the ages of 50-80 with symptomatic, radiographic knee OA scheduled to undergo their first total knee arthroplasty for severely decompensated knee OA. To be included, participants must also have radiographic (Kellgren Lawrence grade 2-4) OA in the contralateral knee that is either partially (group 1; mild to moderate knee pain), or well compensated (group 2;

minimal to no knee pain); (n=16 per group). Pain (lower score = worse pain) will be assessed in each knee using the Knee Osteoarthritis Outcome Score (KOOS) pain subscale, with thresholds set at >80 (minimal to none), 50-70 (mild to moderate), and <40 (severe). Exclusion criteria will include prior knee arthroplasty in either knee, OA in the contralateral knee that is decompensated (severe pain), knee injection with any agent within the last 6 months, taking prednisone or disease-modifying anti-rheumatic medications, or another rheumatologic disease.
Eligible patients will complete patient-reported measures of pain and function for both knees, performance-based measures of function, assessment of movement-evoked pain using a 3-minute step test, and a standardized ultrasound of both knees to assess synovial inflammation. During knee replacement surgery, small biopsies of synovial tissue (1x1 cm) from the lateral recess will be collected from both knees (including the non-surgical knee) through a small incision on the lateral thigh similar to a skin biopsy. Demographics and confounding factors including age, sex, body composition, and comorbid medical conditions and medications will also be collected.
Primary outcomes will be assessed using synovial histopathology and bulk RNA sequencing on RNA samples isolated from synovial tissues. Contrasts between partially/well compensated and decompensated OA knees will be performed using mixed-effects models, with and without adjustment for confounding factors.

Expected Outcomes

Although contrasting OA vs healthy joints would identify OA-related processes, it will not permit differentiation between pathophysiologic and compensatory (protective) processes. Instead, we will investigate contrasts between pairs of OA knees from the same patient with different states of compensation. This will allow us to differentiate between regenerative and pathophysiologic processes, while controlling for patient-specific sources of variability and processes that are generally active in OA.
Contrasting decompensated vs well-compensated will identify 1) regenerative processes that increase in well-compensated OA, and 2) pathophysiologic processes that increase in decompensated OA. We expect that well-compensated OA will be associated with increased synovial tissue vascularization (histopathology) and anabolic processes (RNA sequencing) such as fibroblast growth factor signaling, angiogenesis, and cellular respiration, whereas decompensated OA will be associated with increased fibrosis, perivascular edema, lining hyperplasia, and inflammatory cell infiltrates (histopathology), and pro-inflammatory processes (RNA sequencing) such as Toll-like receptor, proteinase-activated receptor, and complement signaling.
Contrasting partially compensated vs decompensated OA will identify 1) regenerative processes that increase in partially compensated OA that may be insufficient to maintain full compensation, and 2) pathophysiologic processes that increase in decompensated OA that may inhibit regenerative processes. We expect that the histopathology and RNA sequencing results of these contrasts will be similar and support the findings from well compensated OA, with some results unique to each state.
Further, comparing our results between contrast experiments will identify regenerative mechanisms uniquely associated with well compensated OA, and pathophysiologic mechanisms unique to decompensated OA that may inhibit regenerative processes. Collectively, these experiments will produce a list of high-probability candidate targets for enhancing and/or disinhibiting joint-regenerative mechanisms to treat OA. We will validate these candidates directly in future studies.

Research Environment – Description of the number of research personnel, primary location of research, size of lab, etc

The student will be learning in an engaging clinical environment and research environment.
In the clinic, the student will be supervised by Dr. Brent Lanting at University Hospital, where participant recruitment, surgery, and biopsy collection will take place. This environment will provide experience recruiting patients to the study, and experience participating in the clinic and OR setting.
In the laboratory, the student will experience an excellent research training program as part of the Appleton Lab (focused on synovial joint research). This part of the project will take place in the Dental Sciences Building at Western University (adjacent to University Hospital). Several supportive team members will be conducting research alongside the student, including Dr. Appleton, lab technicians, a postdoctoral fellow, and several graduate and undergraduate students. All tissue processing and data analysis will take place at the Appleton Lab.

Names and titles of other individuals who will be involved with the research project?

Dr. Brent Lanting, Associate Professor, Department of Surgery (Orthopedics)	
Can this project be done remotely?	No
Duration of Project	Two Summers

Expected Objectives/Accomplishments for Student for Year 1?
The first summer will be dedicated to patient recruitment and sample collection. A total of 32 patients are targeted for recruitment, which can be achieved in 2-3 months under normal conditions in our prior experience running multiple parallel cohort studies of this nature. Data collection from the biological samples (histopathological processing, grading, RNA sequencing) will also be well-underway, with final data collection from the first 50% of samples.

Expected Objectives/Accomplishments for Student for Year 2?
The second summer will be dedicated to collecting the remaining data collection from histopathological tissue sections, pre- and post-processing of RNA sequencing data, and final data analysis and write-up into manuscript format.

PART III - Certifications

If the project will require any certification approvals from one or more of the following offices, please check the appropriate box below.	- Human Ethics - Biohazard
Human Ethics: If you have the protocol information, please enter it below (or enter the status of the approval).	REB will be submitted and approved before summer 2024
Biohazard: If you have the protocol information, please enter it below (or enter the status of the approval).	BIO-UWO-0340

Note: certification approval should be obtained prior to the start of the summer. Projects without this approval will not be a priority for funding.