HUMAN KNEE TISSUE PROCUREMENT, RESECTION AND PROCESSING FOR MULTIMODAL ANALYSES

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1.0 PURPOSE AND GENERAL CONSIDERATIONS

We describe human knee tissue procurement, resection and allocation for multimodal analyses. The protocol is focused on entire knees that are recovered by Organ Procurement Organizations and parts of the protocol are also applicable for human joint tissues that are removed during surgery on the knee or other joints. For entire knees, samples of all joint tissues are resected to allow analysis of 'the knee as an organ'. Samples of each joint tissue are aliquoted for different preservation methods to enable a broad spectrum of analyses including histology, immunohistochemistry, transcriptomics (bulk, single nuclei and spatial) and other omics technologies.

The order of the tissue resection was chosen to allow photographic documentation of the macroscopic appearance of the tissues and allow subsequent macroscopic scoring.

Completion of the protocol for both knees requires a minimum of four trained staff members and takes approximately 5 hours.

2.0 DEFINITIONS AND ABBREVIATIONS

2.1 **ACL** = Anterior Cruciate Ligament
2.2 **COD** = Cause of Death
2.3 **FFPE =** Formalin-Fixed Paraffin-Embedded
2.4 **Gastro** = Gastrocnemius Muscle
2.5 **IPFP** = Infrapatellar Fat Pad
2.6 **IPR** = Infrapatellar region
2.7 **Lat** = Lateral
2.8 **LCL** = Lateral Collateral Ligament t
2.9 **Macro** = Macroscopic
2.10 **MCL** = Medial Collateral Ligamen
2.11 **Med** = Medial
2.12 **NWB** = Non-Weight Bearing
2.13 **OCT** = Cryo-Histology Blocks
2.14 **OPO** = Organ Procurement Organization
2.15 **PCL** = Posterior Cruciate Ligament
2.16 **Post** = Posterior
2.17 **PPE** = Personal Protective Equipment
2.18 **PT** = Patellar Tendon
2.19 **QT** = Quadriceps Tendon
2.20 **RT** = Room Temperature
2.21 **SPR** = Suprapatellar region
2.22 **SRI =** Scripps Research Institute
2.23 **ST** = Semitendinosus Muscle
2.24 **SYN** = Synovium
2.25 **TOD** = Time of Death
2.26 **WB** = Weight Bearing

2.27 **Z-Fix** = Zinc Buffered Formalin

3.0 ENVIRONMENTAL HEALTH AND SAFETY

The following guidelines are for knee tissue resection and processing at SRI.

3.1 SRI EH&S requires all staff to have annual Bloodborne Pathogens (BBP) Training.

3.2 Training for prevention of surgical injuries according to recommendations of the International Sharps Injury Prevention Society.

3.3 SRI staff are wearing PPE, including surgical gown, hair net, shoe covers, facemask with shield, sterile gloves, doubled on dominant hand when harvesting, cut resistant gloves (NoCry brand).

4.0 TISSUE RECOVERY SITES

Organ Procurement Organizations (OPO)

5.0 TISSUE RECOVERY TEAMS

OPO Surgical Staff

6.0 KNEE RECOVERY NOTIFICATION

6.1 OPO notifies SRI team about potential donor and review of donor information:

- OPO Donor ID
- Age
- Sex
- COD
- TOD
- Medical history
- Medication history
- Serology report
- Expected time of knee recovery and delivery at SRI

6.2 SRI Team reviews information and approves/declines knee recovery.

6.3 Exclusion criteria include a history of knee arthroplasty, inflammatory arthritis, sepsis, immobility (complete bed rest) for more than 2 weeks. Serologies positive for Hepatitis B, Hepatitis C, or HIV.

7.0 DONOR IDENTIFICATION NUMBER

Each donor is assigned an ID (year-consecutive donor number 24-123) which is used to record all donor data and biospecimens. All tubes containing the biospecimens are labeled with the donor ID.

8.0 KNEE RECOVERY

The OPO surgical team resects both knees en bloc, 10 cm above and below the joint line.
A ring of skin (3 cm wide) at the joint line that encompasses the entire circumference of the knee is left intact. The remaining skin is removed.
The knees are wrapped in sterile gauze that is soaked in sterile saline and placed in tightly sealed sterile plastic bags.

Bags are placed on wet ice in a cooler for transport by courier to SRI.

9.0 KNEE PHOTOGRAPHS

The knees are removed from the bags and placed on a surgically draped harvesting bench. Photographs are taken from the anterior, medial, lateral and posterior views (Figure 1).

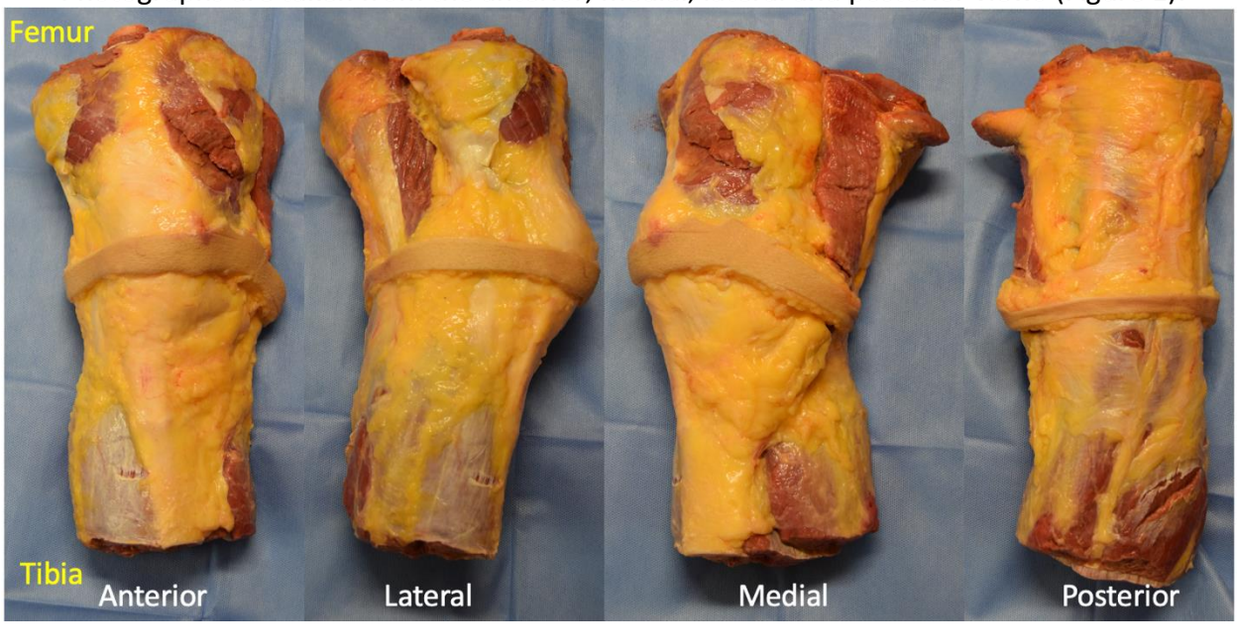

Figure 1. Knees en bloc

10.0 SYNOVIAL FLUID COLLECTION

Synovial fluid is collected from each knee through a superolateral approach (Figure 2).

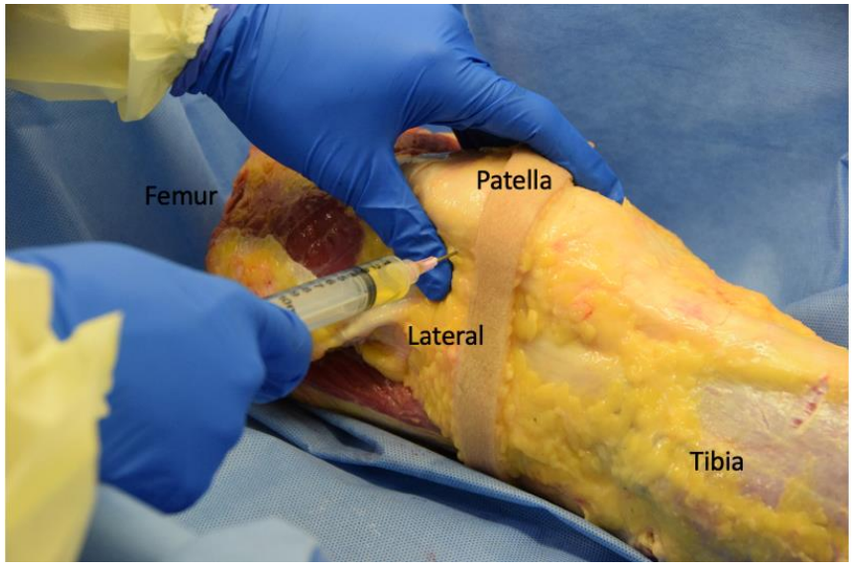

Figure 2. Synovial fluid aspiration

The knee is flexed and extended ten times to reduce the fluid viscosity and to distribute more evenly into the joint space. The surgeon's non-dominant hand is placed on the anterior knee and pressure is applied at the patella and PT and the medial and lateral sides. This allows access to the suprapatellar pouch region.

A sterile 18G needle is attached to a sterile 10mL syringe, then placed into the suprapatellar pouch, just below the superior aspect of the patella. This is inferior, posterior, and lateral to the lateral femoral condyle, taking care not to damage the articular cartilage.
The amount of synovial fluid varies from donor-to-donor, ranging from 0.5-3ml in young healthy knees to >5ml in knees with osteoarthritis.
The synovial fluid is aspirated into the syringe and transferred into a 15mL centrifuge tube. The color, presence of blood and debris in synovial fluid are recorded. The synovial fluid is centrifuged at 1800 x g for 5 minutes. The clear supernatant is transferred into 2mL tubes, taking care to remove as much fluid as possible while not disrupting any pellet (from cells or other debris) at the bottom of the centrifuge tube. Aliquots (up to 2ml volume each) are stored in 2mL Eppendorf tubes at -80ºC.

11.0 OPENING THE KNEE JOINT AND CAPSULE

11.1 With a scalpel, the QT is cut close to the insertion site at the superior margin of the patella. Tension is created on the PT by pulling the superior region of the patella towards the femur.
Then the capsule around the medial and lateral sides of patella (tracing with scalpel) is cut along either side of the PT using smooth cuts to keep all tissues intact.
The synovium and capsule remain *in situ* for photographs (Figure 3).
11.2 Photographs of IPFP (Figure 3a) and Syn Med (Figure 3b) in situ.
Two photographs are taken of the opened joint: the knee joint with IPFP visible (patella with PT pulled away from the joint), medial synovium (syn med). Using forceps, the entire medial synovium is exposed for this photo (Figure 3).

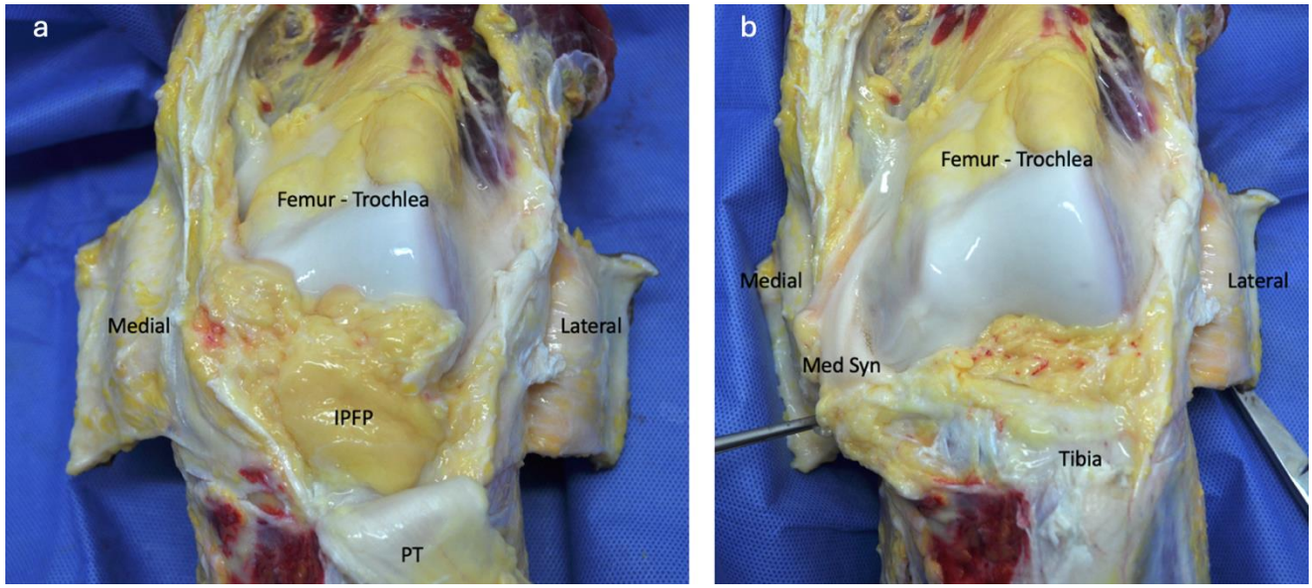

Figure 3. Photos of IPFP and Synovium

12.0 IPFP

12.1 Using a scalpel, the IPFP is separated from the PT and the bursa closer to the tibial end of the PT is localized (Figure 3a). Creating tension on the PT with the forceps, the scalpel is placed through the bursa and against the PT. The IPFP is resected from the PT, moving the scalpel from the tibial end towards the patella end of the PT. The IPFP will remain on the

knee joint, located on the tibia and the joint space. The patella is pulled away from the joint to expose the anterior aspect of the IPFP (Figure 3b).

12.2 Photograph of the anterior IPFP in situ.

12.3 A scalpel is used to cut along the tibia and resect the IPFP from the bone. On the lateral and medial aspects of the IPFP, the IPFP is carefully resected while retaining the peri-IPFP synovium. This same technique is applied to the superior region of the IPFP to retain supero-IPFP synovium. Orientation must be noted. The resected IPFP is placed in a Petri dish with the posterior side on the dish, with Superior, Inferior, Medial and Lateral marked on the petri dish.

12.4 Photographs of the IPFP: IPFP is placed onto the photo station glass with the posterior side (which faces the joint space) on the glass and anterior side facing the camera (Figure 4a). A scale is placed at the bottom of the photo frame and the superior region of the IPFP is oriented towards the top of the frame. The photo is taken while turning the IPFP so that the anterior side of the tissue is facing the camera. The superior region is oriented towards the top of the photo frame and image the tissue. The tissue is returned to the petri dish in its original position; posterior side of the IPFP on the petri dish.

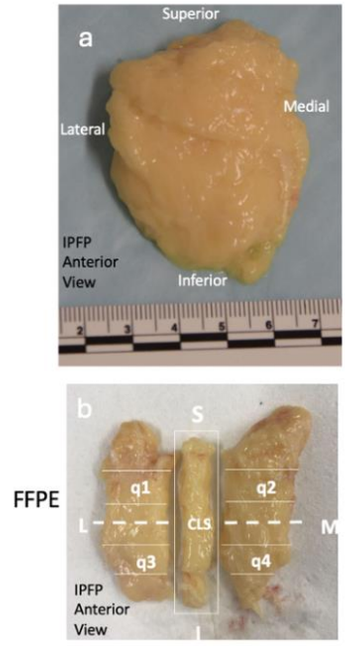

Figure 4. Infrapatellar fat pad

12.5 IPFP allocation

12.5.1 A central longitudinal section from the superior to inferior part of the IPFP is collected for FFPE (Figure 4b). Tissue marking dye (Cancer Diagnostics Inc) is used to mark the superior aspect of the tissue. The tissue is placed into a histology cassette to maintain orientation and the cassette is placed in an 8oz sample container with 160mL of Z-Fix.

12.5.2 The surrounding tissue is divided into four quadrants and collected for FFPE: q1 superior lateral, q2 superior medial, q3 inferior lateral, and q4 inferior medial. These pieces of tissue are placed in cassettes to maintain orientation. They are placed into the sample container described in 12.4.2.

12.5.3 The remaining tissue is combined and divided up for preservation:

> in OCT for histology

> in AllProtect (≥ 150mg in 5ml eppendorf tube with 3 pumps of AllProtect) for RNA/protein isolation

> Snapfrozen (≥500mg) in 2mL cryovials for nuclei isolation and other applications

> Extra tissue can be used for cell isolation.

12.6 IPFP macroscopic scoring

IPFP macroscopic scores are completed using the photos taken in step **12.4**. The scoring system used for macroscopic grading is in referenced in PMID: 20864026.

13.0 PATELLA

13.1 Resection of the patella from the PT (Figure 5a).

13.2 Photograph of the patella

The patella is placed onto the photo station glass with the cartilage side facing the camera. A scale is placed at the bottom of the photo frame and the inferior point of the patella faces towards the top of the frame to take a photograph (Figure 5b).

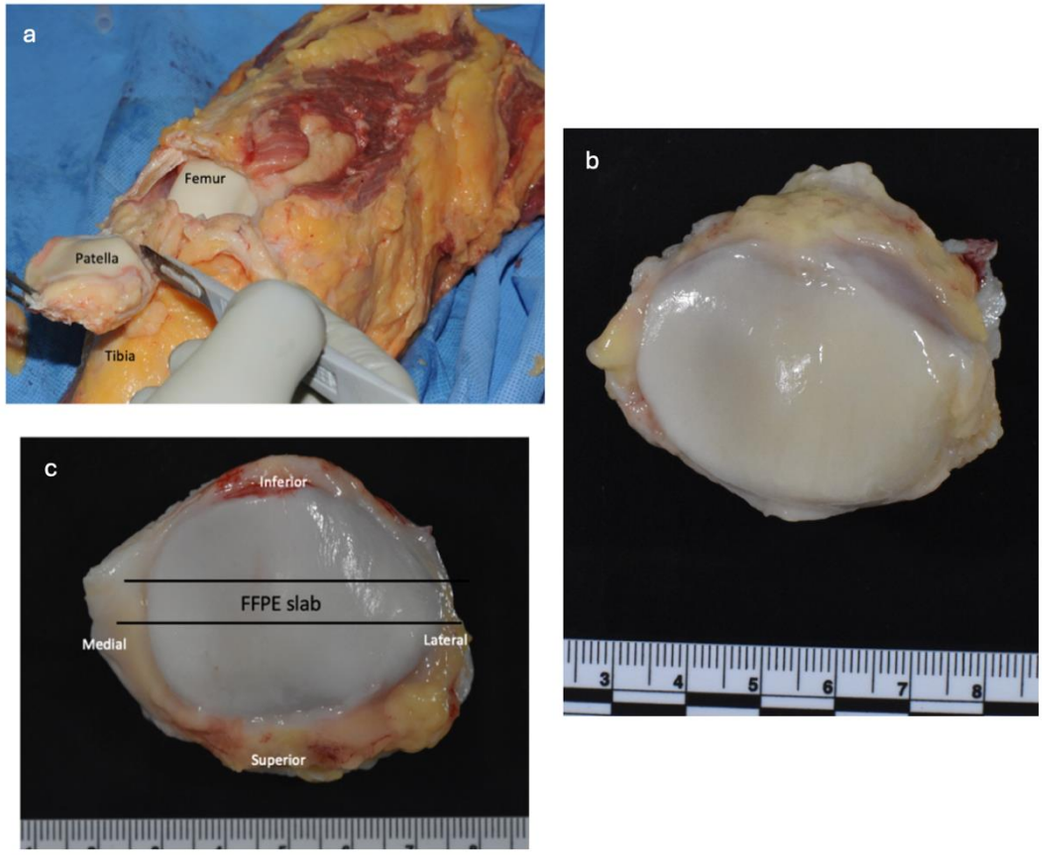

Figure 5. Patella resection

13.3 Patella slab for histology: A pathology saw is used to cut out a 2mm wide slab across the center of the patella from its medial to lateral aspects (Figure 5c). This slab is placed into 4oz sample container in 60ml of Z-Fix.

13.4 FFPE sample preparation after fixation and decalcification: From medial to lateral, 3 pieces #1-3 are cut. Away from the center of the slab and slightly towards the medial side is section

#1. The remaining patella is cut into half for pieces #2 and #3. Trabecular bone is trimmed to a thickness of 2-3mm.

14.0 PATELLAR TENDON

14.1 The PT is resected from the tibial tuberosity (Figure 6a) and placed into a petri dish for tissue allocation (Figure 6b).

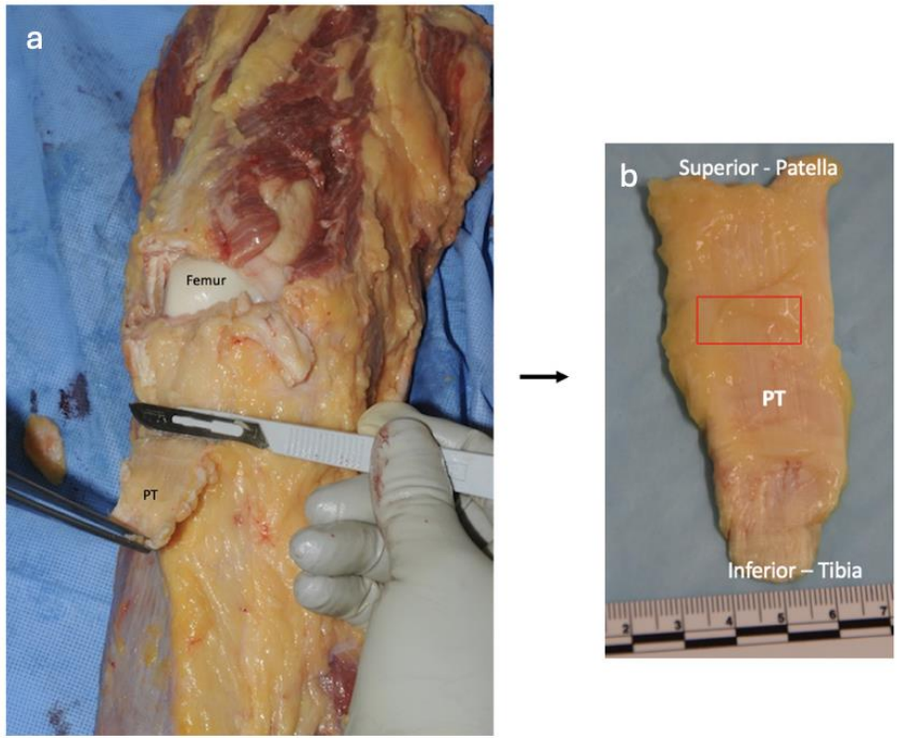

Figure 6. Patellar tendon resection

14.2 The central portion of the PT (size) is resected placed into a scintillation vial with 17ml of Z-Fix for FFPE processing.

14.3 Remaining tissue is divided into equal halves for snapfreezing in 2mL cryovials and placed in a 5ml eppendorf tube with 3 pumps of AllProtect.

15.0 MACROSCOPIC GRADING OF CARTILAGE SURFACES

The modified Outerbridge grading system (PMID: 14167619) is used to assign scores to the cartilage surfaces of the patella, femoral condyles, and tibia. The patella is divided into 9 regions, the femoral trochlear region into 3 regions and each femoral condyle into 9 regions. The tibia is divided into 18 regions (9 on the medial plateau and 9 on the lateral plateau). Scores are assigned to each of the 48 regions.

Each region is assigned a score of 1-4.

Score 1: Normal – has intact surface
Score 2: Minimum Fibrillation – fibrillation and/or softening
Score 3: Overt Fibrillation – fissuring
Score 4: Erosion - to bone

The scores for each of the 48 cartilage regions are recorded on the Gross Morphological Assessment Sheet, a template that shows the regions (Figure 7). The values on the sheet are used to record a modified Outerbridge macroscopic scoring of the cartilage tissues from each

knee's patella, femur, and tibia. The Total Knee Cartilage Grade is calculated and written at the top of the sheet. The presence of osteophytes is also recorded.

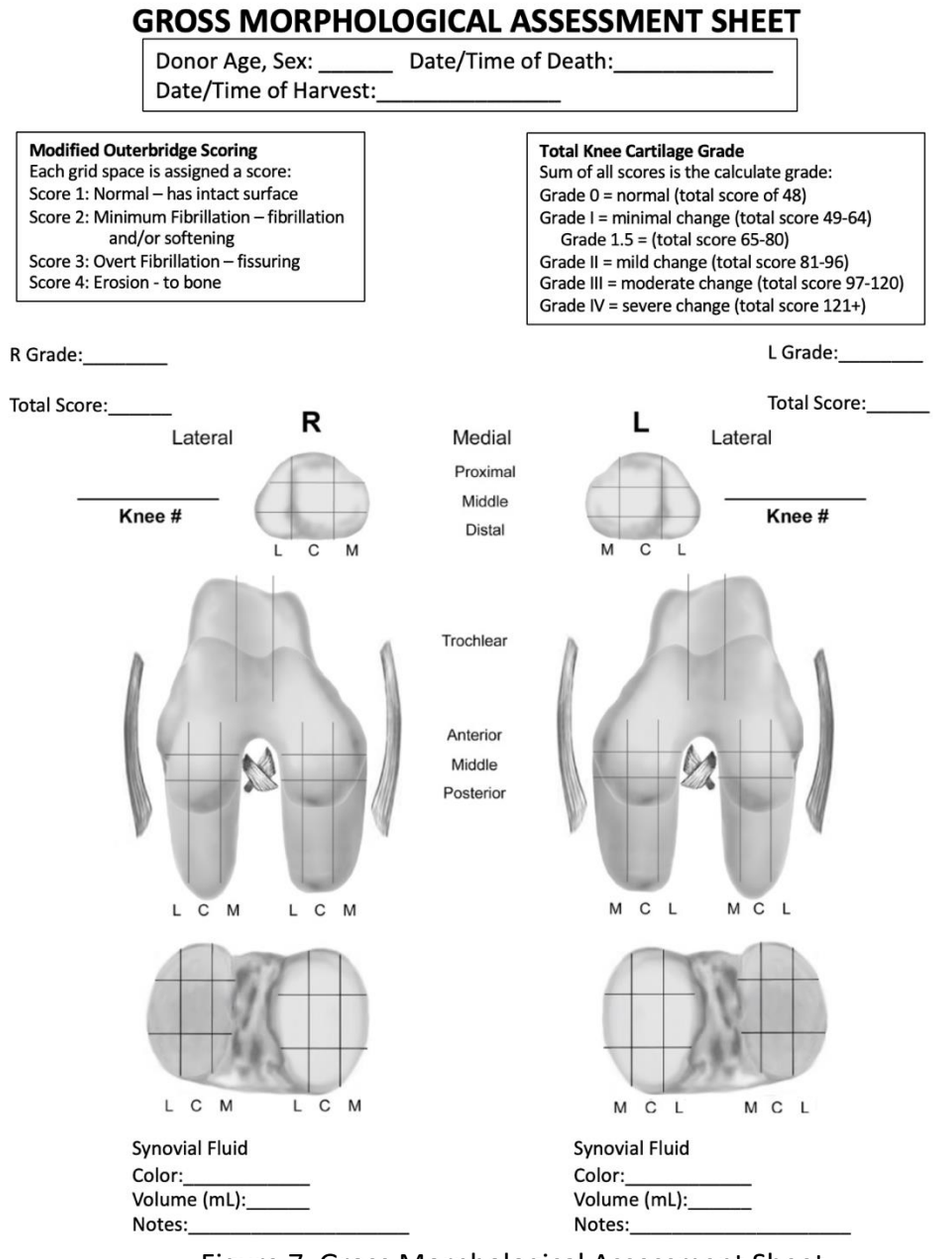

Figure 7. Gross Morphological Assessment Sheet

The sum of all scores is calculated and used to determine the Total Knee Cartilage Grade:

Grade 0 = normal (total score of 48)
Grade I = minimal change (total score 49-64)
Grade 1.5 = (total score 65-80)
Grade II = mild change (total score 81-96)
Grade III = moderate change (total score 97-120)
Grade IV = severe change (total score 121+)

16.0 RESECTING AND PROCESSING THE MEDIAL SYNOVIUM

16.1 The entire medial synovium (Syn med) is resected (Figure 8a) and orientation is retained and noted in a petri dish. This medial region is defined as the medial aspect of the knee where the synovial borders are in line with the 1. superior medial trochlea, 2. down to the attachment site of the medial meniscus, 3. anteriorly where the synovium attached to the medial patella/PT, and 4. posteriorly to include the medial gutter. The outermost tissue and capsule are placed into the petri dish, synovium facing up.

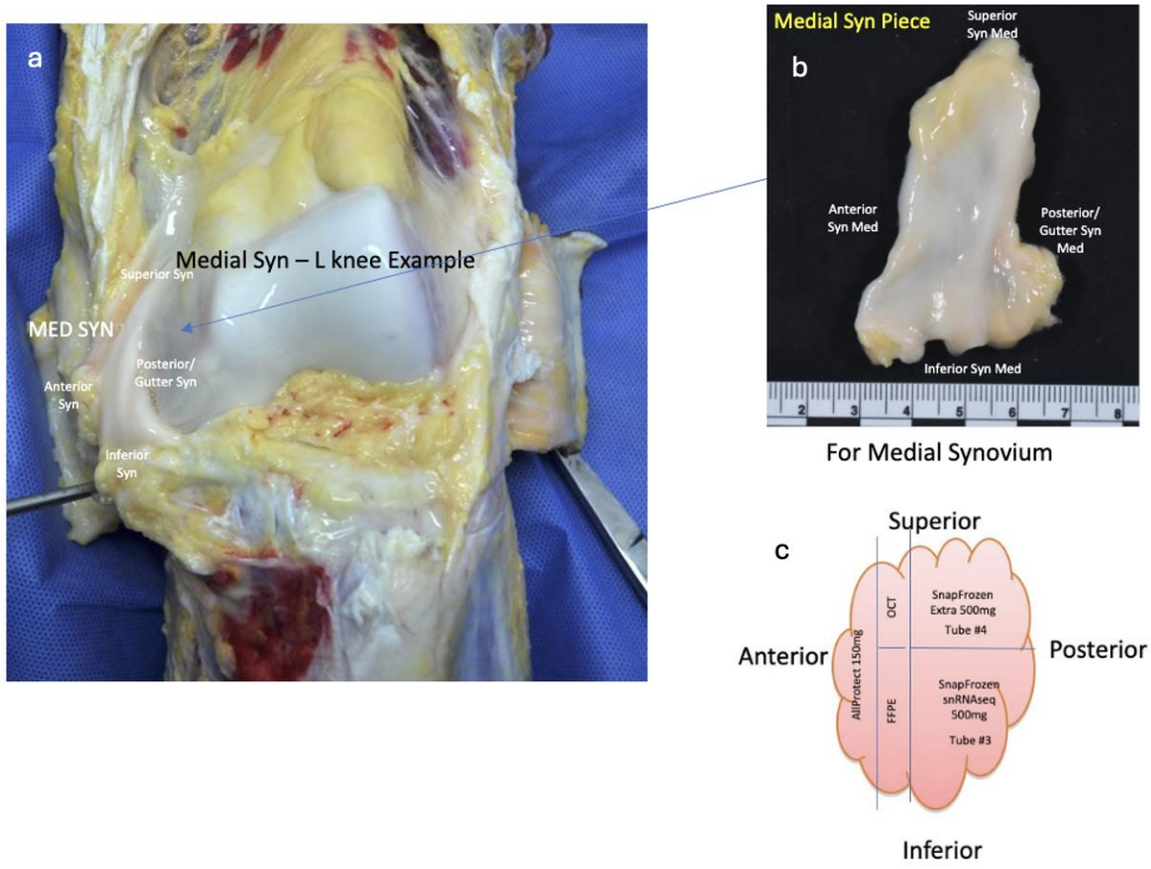

Figure 8. Medial Synovium

16.2 Photograph of the synovium

Syn med is placed onto the photo station glass with the capsule side on the glass and synovium side facing the camera (Figure 8b). A scale is placed at the bottom of the photo frame and the superior region of the syn med tissue is oriented towards the top of the frame. The photograph is taken, and the tissue returned to the petri dish in its original position.

16.3 Syn med allocation

The synovium tissue is oriented with superior, inferior, anterior (location when knee joint is unopened), and posterior (medial gutter along femur) (Figure 8c).

16.3.1 > 150mg AllProtect sample is collected from the most anterior aspect in 5ml Eppendorf tube with 3 pumps of AllProtect.

16.3.2 Tissue for OCT histology is taken from the supracentral region.

16.3.3 A FFPE sample is collected from the infracentral region. This is placed into a histology cassette to maintain orientation and the cassette is put into a 4oz sample container in 60mL of Z-Fix.
16.3.4 >500mg snapfrozen tissue is collected from the posterior region in 2ml cryovials.
16.4 Syn med macroscopic scoring
Syn med macroscopic scores are completed using the photos taken in step **16.2**. The scoring system used for macroscopic grading is in referenced in PMID: 20864026.

17.0 RESECTING AND PROCESSING THE SYNOVIUM FROM LATERAL, INFRAPATELLAR AND SUPRAPATELLAR AREAS

Synovium is also collected from the suprapatellar (superior), infrapatellar (inferior) and lateral regions of the knee (Figure 9).

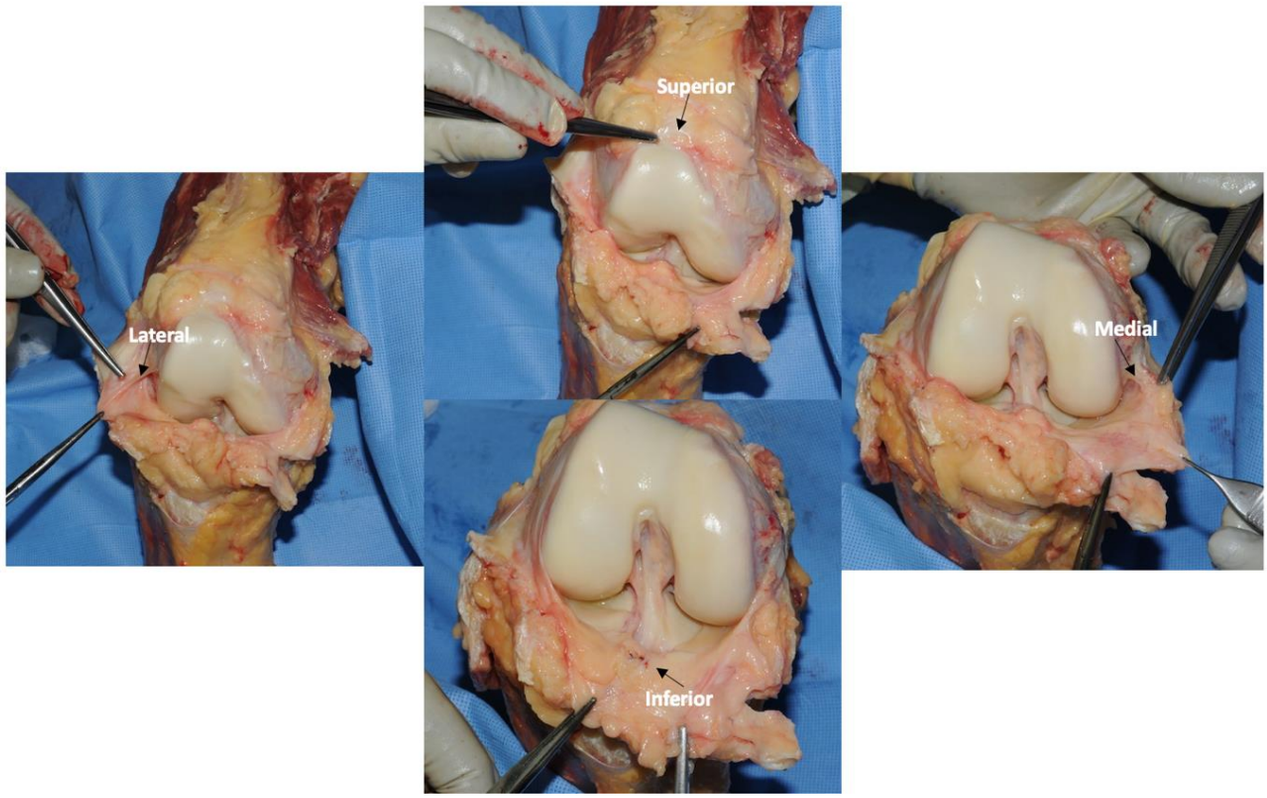

Figure 9. Anatomical locations of synovia

From each region, adjacent tissue slices are used for:
17.1 >150mg AllProtect samples are placed in 5ml Eppendorf tubes with 3 pumps of AllProtect.
17.2 An FFPE sample is collected and placed into a histology cassette to maintain orientation and the cassette is put into Z-Fix in the same container described in 16.3.3.
17.3 Extra synovial tissue can be used for cell isolation.

18.0 SKIN-JOINT CAPSULE-SYNOVIUM BIOPSY MEDIAL/LATERAL

18.1 A #11 scalpel is used to cut out a biopsy at the joint line from the medial (Figure 10) and lateral sides of the knee, which includes skin, joint capsule and synovium. The goal is to capture an entire core to visualize nerves in all layers from skin to the synovial lining.

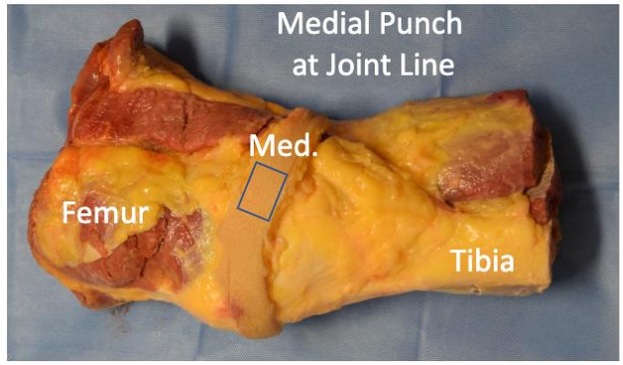

Figure 10. Medial Punch

18.2 Each biopsy is placed in a separate 4oz sample container with 60ml Z-Fix.

19.0 QUADRICEPS TENDON

The slices are cut into 3 similar size (30mmx10mm) pieces for

19.1 FFPE – the sample is placed into a scintillation vial with 25ml of Z-Fix.

19.2 >500mg snapfrozen tissue is collected in 2ml cryovials.

19.3 Allprotect – the sample is placed into a 5ml Eppendorf tube with 3 pumps of AllProtect.

20.0 SEMITENDINOSUS TENDON

Slices (30mm x10mm wide) are cut at the transition sites

20.1 FFPE – the sample is placed into a scintillation vial with 25ml of Z-Fix.

20.2 >500mg snapfrozen tissue is collected in 2ml cryovials.

20.3 Allprotect – the sample is placed into a 5ml Eppendorf tube with 3 pumps of AllProtect.

21.0 GASTROCNEMIUS MUSCLE

21.1 FFPE – the sample is placed into a scintillation vial with 25ml of Z-Fix.

21.2 >500mg snapfrozen tissue is collected in 2ml cryovials.

21.3 Allprotect – the sample is placed into a 5ml Eppendorf tube with 3 pumps of AllProtect.

22.0 MCL AND LCL

MCL and LCL are isolated by tracing the ligament boarders with a #11 scalpel (Figure 11).

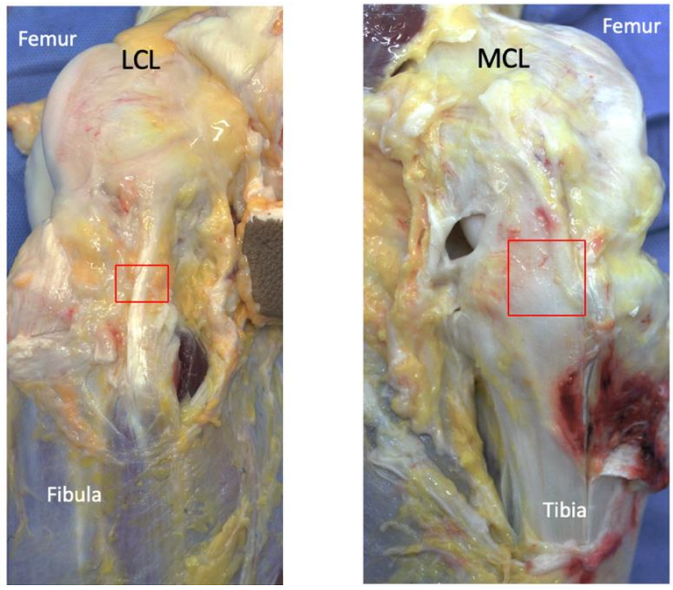

Figure 11. Collecting the mid-substance of ligaments at the joint line

The mid-substance is then resected and collected for:

22.1 FFPE samples are taken from the mid-substance of the ligaments, at the joint line. Samples are placed into 17ml of Z-Fix in scintillation vials.

Remaining tissues are divided up for:

22.2 Snapfrozen in 2ml cryovials

22.3 AllProtect samples are placed in 5ml Eppendorf tubes with 3 pumps of AllProtect.

23.0 DISARTICULATING THE FEMUR

23.1 Excess soft tissues are removed while keeping the posterior capsule intact from around the knee and cortical bones. This includes muscle, fat, synovium, tendons, vessels, etc.

The knee is flexed 90° to expose the anterior femur and ACL. A photo of knee and exposed ACL is taken (Figure 12).

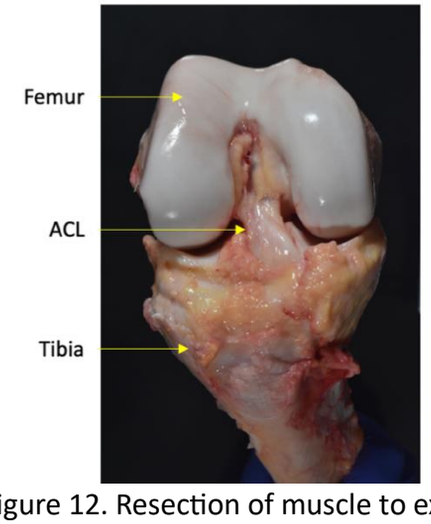

Figure 12. Resection of muscle to expose ACL

23.2 ACL and PCL are resected from femoral insertions

With #11 scalpel and forceps the medial and lateral capsule are released from the femur (Figure 13a). Do not apply excessive force to ACL and PCL and create tension by bending the femur (flexed 90°). Using the scalpel and tracing along the condylar notch the ligaments are resected (Figure 13b). The posterior capsule must remain intact and will be exposed once the ACL and PCL are fully resected.

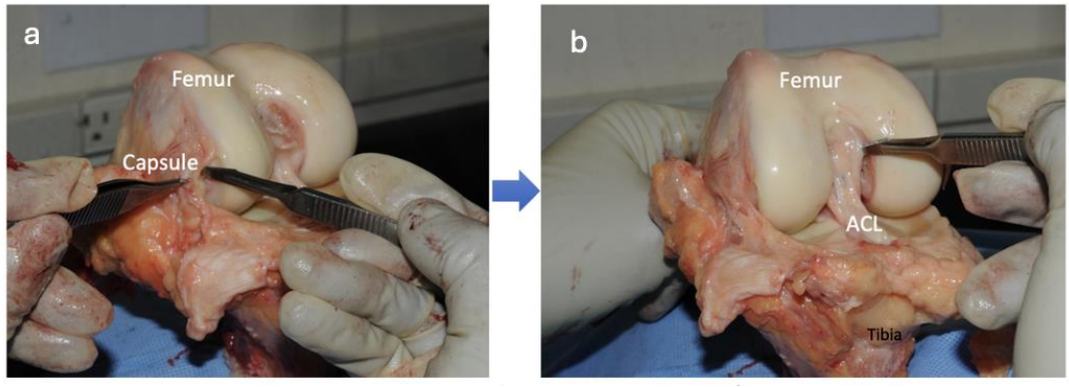

Figure 13. ACL and PCL resection at femur

24.0 SKIN-JOINT-CAPSULE-SYNOVIUM BIOPSY POSTERIOR

24.1 A #11 scalpel is used to core out a biopsy at the joint line from the posterior side of the knee, which will include skin, joint capsule and synovium (Figure 14a & 14b). The goal is to capture an entire core to visualize nerves from all layers from skin into the joint lining. Cutting out the core is easiest when tension is created by lifting the femur and cutting from inside the joint to outside/skin (Figure 14c).

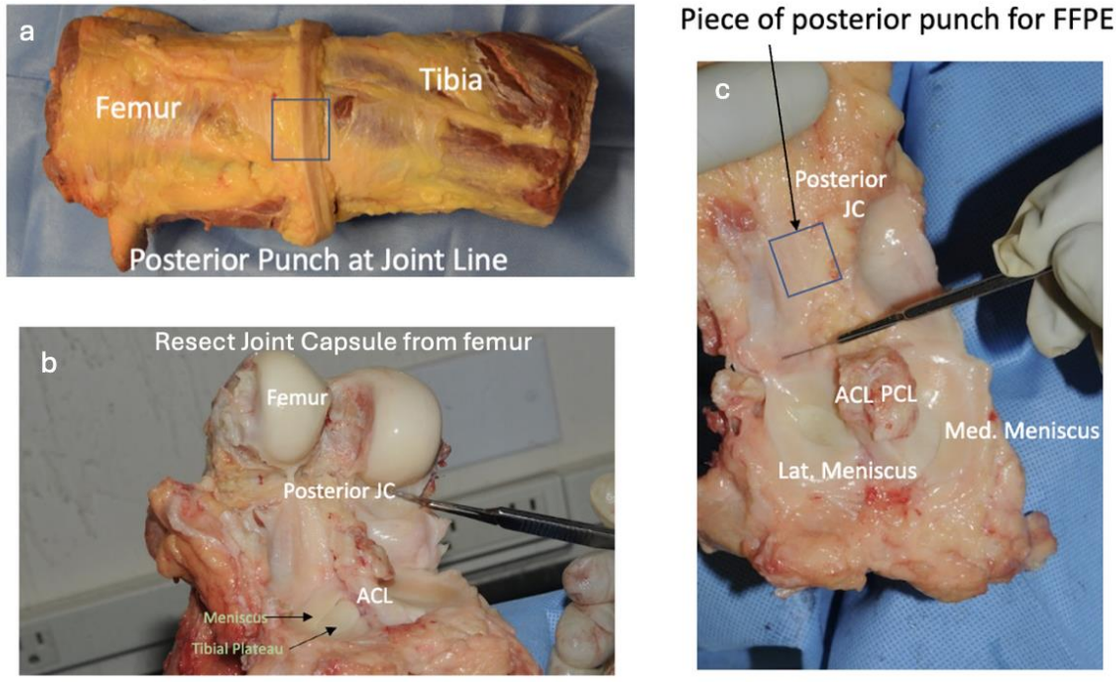

Figure 14. Posterior Punch collection and Joint Capsule (JC) release

24.2 The posterior biopsy is placed in an 8oz sample container with 160ml Z-Fix.

After the punch biopsy has been obtained, excess soft tissue is removed from the femur.

25.0 MACROSCOPIC SCORING AND PHOTOGRAPH OF FEMUR

25.1 At this stage, the entire femur is exposed.

Macroscopic scoring is done using the cartilage (Outerbridge system) of the trochlear region and condyles (21 grid spaces total) and values are recorded on the Gross Morphology Sheet (Figure 7).

25.2 Photographs of the femur are taken at the photo station with femur held in position for the following views: Anterior, Angle Troch1, Angle Troch2, Inferior, Posterior, 45° Medial, Medial, 45°Lateral. A total of 9 photos are taken for each femur (Figure 15).

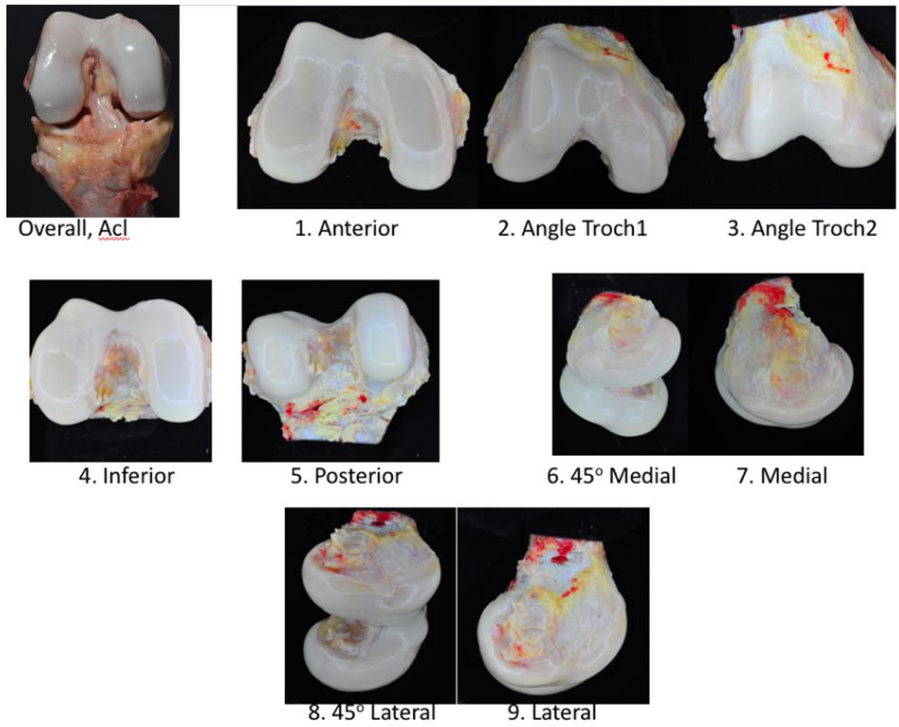

Figure 15. Macro pictures of femur

25.0 FEMUR PROCESSING

26.1 A pathology saw is used to cut osteochondral slabs from the WB Region (approx. 20x30x2.5mm, depending on size of knee) and the NWB Region (approx. 20x25x2.5mm depending on donor) from the medial and lateral condyles (4 slabs total, Figure 16).

From each condyle, a 2mm slice is cut for storage in Z-fix for FFPE and 4mm slabs are taken for tissue allocation. Excess trabecular bone is trimmed away.

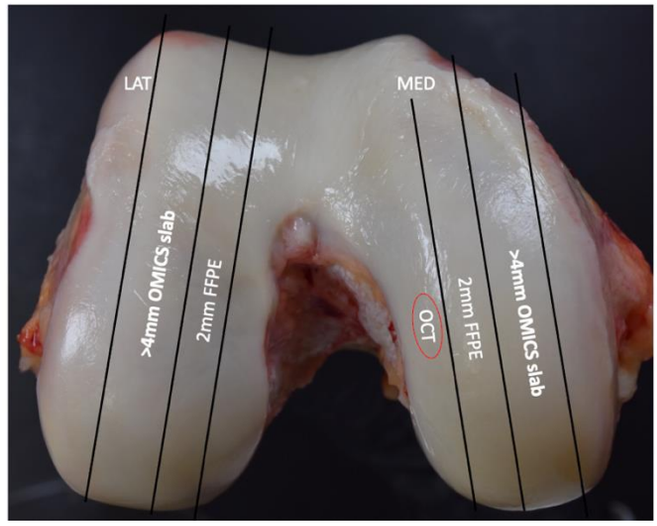

Figure 16. Femoral Condyle – cartilage and osteochondral samples

26.2 Tissue allocation and areas of interest:

26.2.1 OCT full-thickness pieces of cartilage are taken from the medial condyle's weight bearing region from the remaining tissue after slabs are removed (Figure 16).

26.2.2 2mm slab - placed into 8oz sample container with 160ml Z-Fix for FFPE sample preparation after fixation and decalcification. The slab is cut into 6 sections #1-6 from anterior to posterior (more curved), pieces are about 15mm long, 2-3mm thick. Trabecular bone is trimmed down to about 2mm thickness.

26.2.3 4mm slab – is separated into WB and the posterior NWB regions (Figure 17a). Each of the WB and NWB regions are collected separately for different downstream methods/analyses.

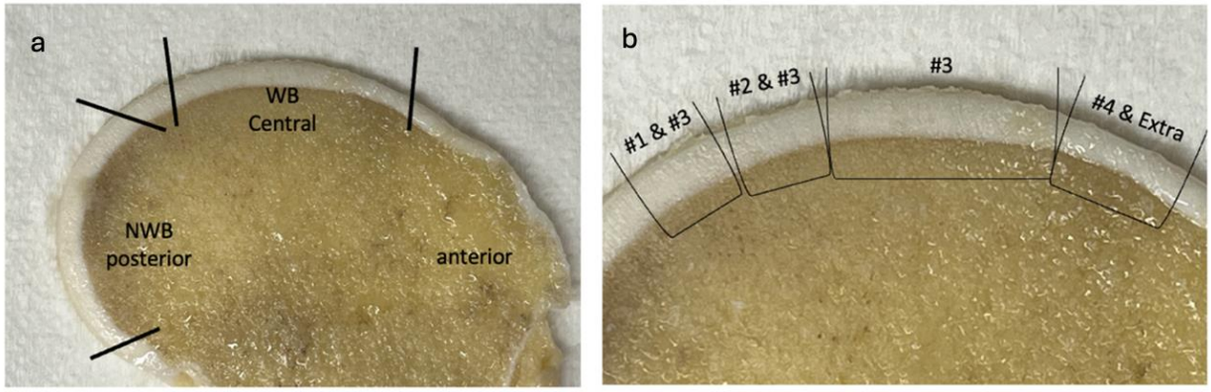

Figure 17. Femoral Condyles – osteochondral samples

Figure 17b (higher magnification of WB and NWB regions with allocations for different analyses):

26.2.3a Spatial transcriptomics [#1 & #2] - n=2 2mmx2mm osteochondral tissues are put into 1.5ml Eppendorf tubes in 1ml Z-Fix for FFPE.

26.2.3b Snapfrozen

[#3] >500mg of osteochondral tissue is collected per tube, cartilage and bone (subchondral with a 2mm layer of trabecular bone) are separated and placed into the same 2ml cryovial tubes for storage.

[#4] 150mg of osteochondral tissue is collected for bulk RNAseq

[Extra] Leftover osteochondral tissue is collected and banked.

26.2.4 Remaining cartilage can be shaved from femur bone for cell isolation.

27.0 ACL AND PCL

27.1 Prior to ACL/PCL resection, a top view photograph of tibia with ACL, PCL and menisci is taken on the photo station. This image shows the menisci and ligaments still attached to the tibia (Figure 18).

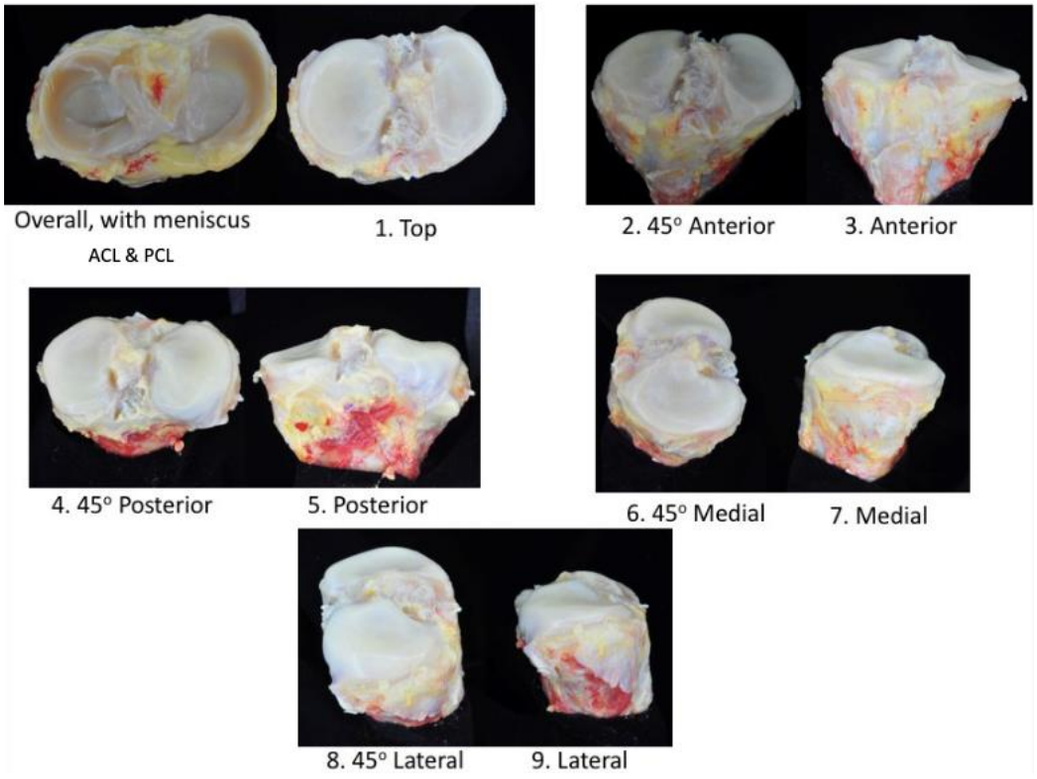

Figure 18. Macro pictures of tibia

27.2 Resecting ACL and PCL

Using a #11 scalpel and forceps, the ACL and PCL are resected. There is a synovial sheath at the femoral aspect of the ligaments. This sheath is held with forceps and not the ligament itself (Figure 19a). Tension is created by pulling up on the sheath and the soft tissue that holds the ACL and PCL together is carefully cut. Create tension on the ACL sheath and use the scalpel to trace along the tibial insertion site to resect the ACL. Then the same technique is used for the PCL resection.

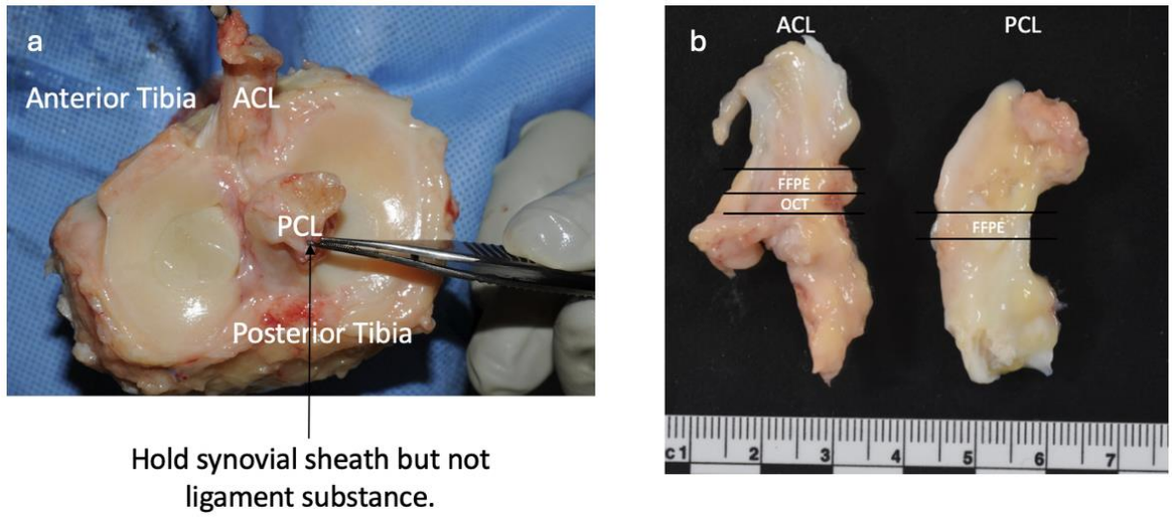

Figure 19. ACL and PCL collection

27.3 Macro image of ACL and PCL

Place the ligaments onto the photo station for macro imaging. Include a scale at the bottom of the photo frame; the ACL (left in the photo frame) and PCL (right in the photo frame) are placed next to each other, and the image is taken (Figure 19b). Place the ligaments into labeled petri dishes for processing.

27.4 ACL and PCL tissue allocation (Figure 19b).

27.4.1 The middle mid-substance of each ligament (4mm thick) is resected on the axial/transverse plane and placed into 17ml of Z-Fix in a scintillation vial for FFPE.

27.4.2 The tissue for OCT is collected from the adjacent tissue as the FFPE sample for ACL only.

27.4.3 >500mg snapfrozen samples are placed in 2ml cryovials for each tissue.

27.4.4 Allprotect – the sample is placed into a 5ml Eppendorf tube with 3 pumps of AllProtect for each tissue.

28.0 MENISCI

When resecting the menisci from the tibia, be careful not to puncture meniscus with forceps or create cuts with the scalpel. Do not damage the tibial cartilage.

28.1 Use forceps to hold and create tension on the synovium attached to the meniscus near the medial meniscus anterior horn. The synovium is released from this attachment site with a #11 scalpel.

While creating tension, the synovium is cut through to the medial meniscus posterior horn site. The same method is applied for the lateral meniscus.

28.2 Macro images of menisci

Both menisci are placed, with the tibial side down, onto the photo station with a scale at the bottom of the photo frame. The first photo is of the top (femoral) side of the menisci (Figure 20a). Both menisci are flipped over so that the second photo is taken of the bottom side (tibial) (Figure 20b)

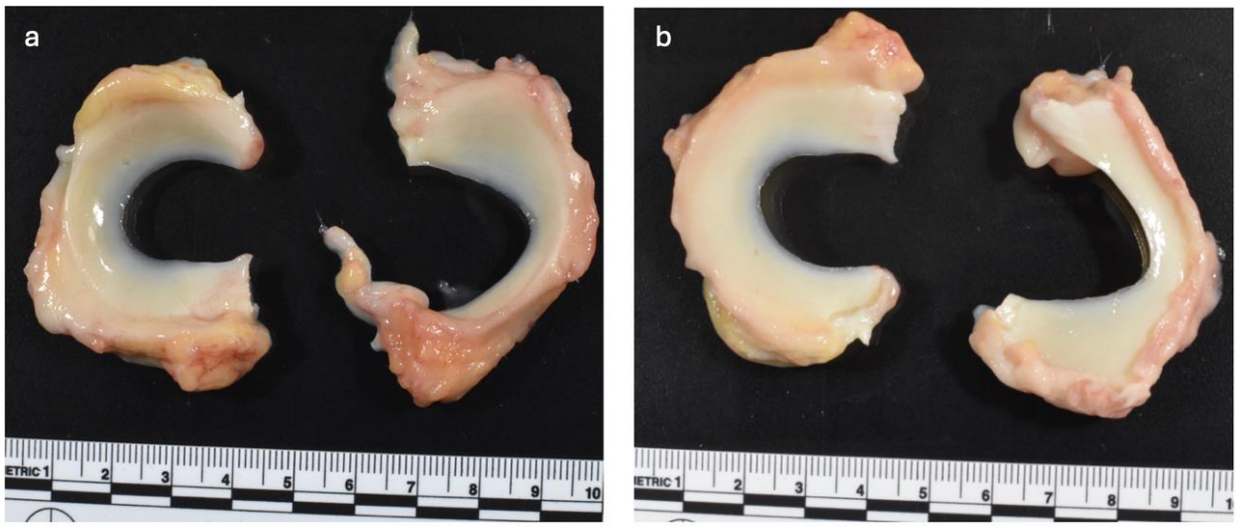

Figure 20. Macroscopic images of menisci

28.3 Tissue allocation (Figure 21)

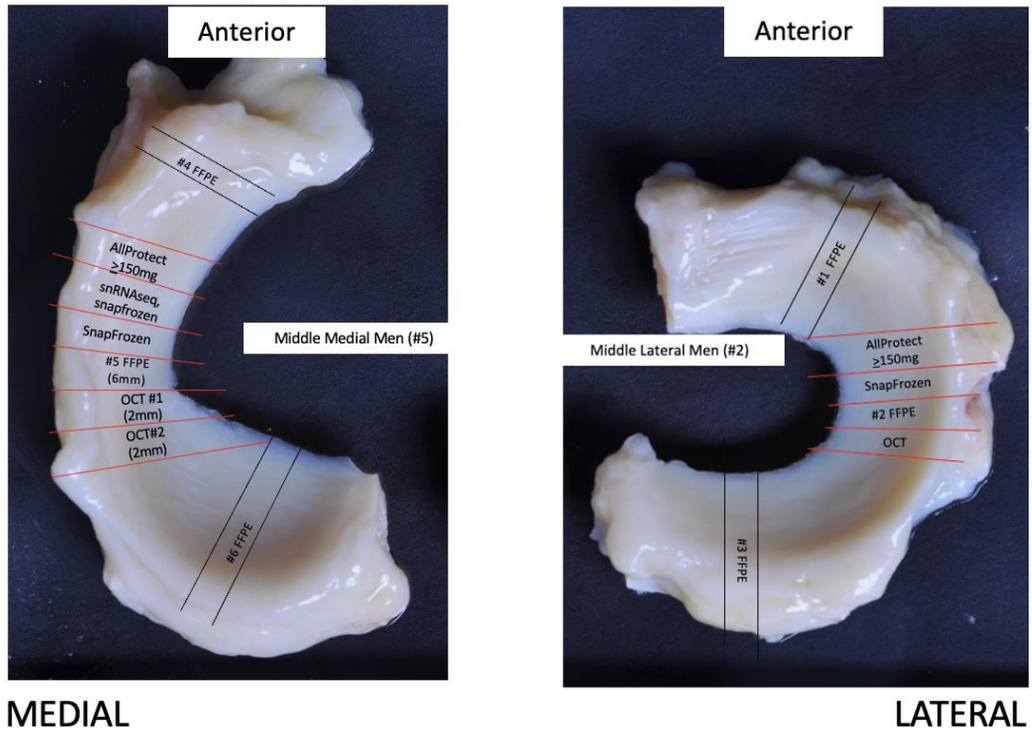

Figure 21. Meniscus tissue allocation

Each meniscus is divided into 3 different regions:
Lateral Meniscus: Anterior (#1), Middle (#2), Posterior (#3)
Medial Meniscus: Anterior (#4), Middle (#5), Posterior (#6)
A slice (4mm) of each is placed in Z-Fix for FFPE histology.
The mid-regions #2 and #5 are stored individually for additional preservation methods:
28.3.1 OCT – adjacent pieces of #2 and #5 are embedded in OCT.

28.3.2 AllProtect – areas #2 and #5 are placed into 5ml Eppendorf tubes with 3 pumps of AllProtect.

28.3.3 Snapfrozen – areas #2 and #5 are snapfrozen and stored in 2ml cryovials.

Remaining tissue can be used for cell isolation.

28.4 Meniscus macroscopic scoring

Meniscus macroscopic scores are completed using the photos taken in step **28.2**. The scoring system used for macroscopic grading is referenced in PMID: 21683797.

29.0 TIBIA

Excess soft tissue is resected from the tibia.

29.1 Macroscopic scoring of tibia

Macroscopic scoring (using the Outerbridge system) of the cartilage of the tibial plateaus (18 grid spaces total) is performed and values are recorded on the Gross Morphology Sheet (Figure 7).

29.2 Photography

Images of the tibia are taken at the photo station and tibia is held and rotated for each photo. A total of 8 photos are taken. These views are captured: Top, 45° Anterior, Anterior, 45° Posterior, Posterior, 45° Medial, Medial, 45° Lateral, and Lateral (Figure 18).

29.3 Tissue Allocation

The pathology saw is used to cut a 2-3mm central osteochondral slab that includes both medial and lateral plateaus (Figure 22a). A mark is made on the medial trabecular bone to indicate the medial side (Figure 22b). The slab is placed into 50ml centrifuge tube with 45ml of Z-Fix for FFPE.

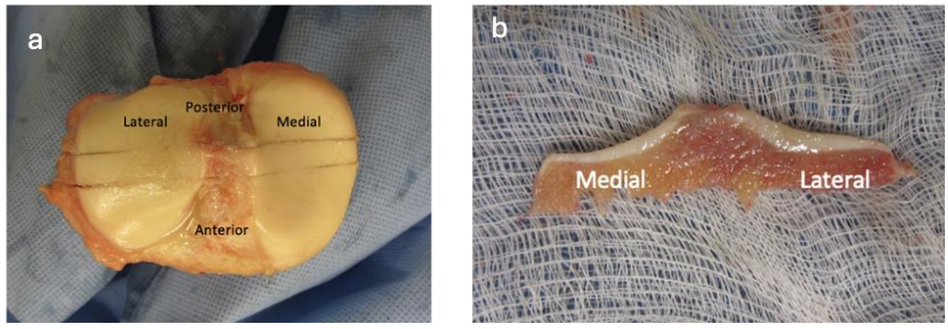

Figure 22. Tibia – osteochondral samples

29.3.1 Remaining cartilage can be shaved from bone for cell isolation.

29.3.2 FFPE sample preparation after fixation and decalcification. The medial and lateral plateaus are separated with a scalpel. two. There will be 4 total pieces from medial to lateral per tibial slab #1 to 4. Trabecular bone is trimmed to 2-3mm thickness.

30.0 SAMPLE STORAGE

30.1 Snapfrozen samples are stored in Liquid Nitrogen.

30.2 OCT cryoblocks are stored at -80C.

30.3 Samples in AllProtect are stored at 4C overnight and then placed at -80°C for long-term storage.

30.4 Samples in Z-Fix are placed onto a rocker at RT for 2 weeks, unless these are used for Spatial Transcriptomics, which are 2x2 mm and fixed for 2 days.

31.0 FFPE HISTOLOGY PROCESSING

31.1 Soft tissues (all except bone)

After 2 weeks of fixation on a rocker, these samples are trimmed, inserted into cassettes and placed onto an automatic tissue processor. Samples are then embedded and sectioned for H&E or Safranin O/Fast Green.

31.2 Calcified tissues (osteochondral)

A minimum ratio of 20:1 decalcification solution to tissue is used.

31.3 Standard FFPE

22% Formic Acid is used for standard FFPE preparation of osteochondral tissues.

This works well for IHC and other standard histological stains.

22% formic acid solution:

10g Sodium Citrate, 780mL deionized water, 220mL Formic Acid

31.4 Standard Decalcification

The osteochondral tissues are decalcified in formic acid at RT on a rocker and checked on progress after one week. A razor blade is used to manually check the decalcification process and to trim down any unnecessary bone tissue. The solution is changed to a fresh solution if the decalcification process is incomplete and checked after a few days.

Once the decalcification process is completed, the tissues are cut to smaller areas of interest (Figure 23), inserted into cassettes and placed onto the automatic tissue processor. Samples are embedded, cut and stained with Safranin O/Fast Green.

31.4.1 Preparing pieces for standard FFPE osteochondral tissues

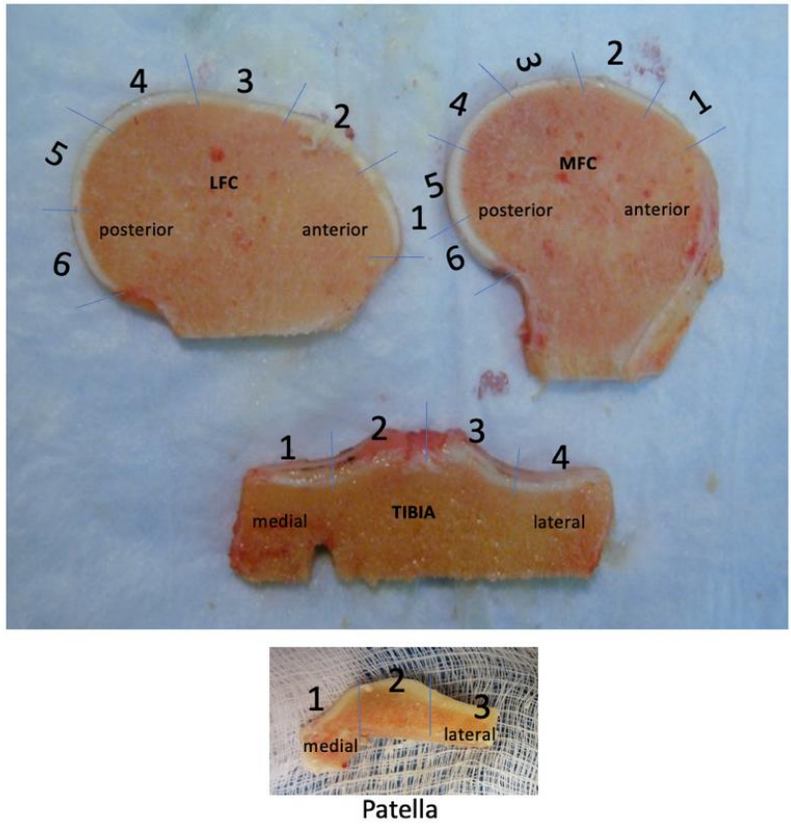

Figure 23. FFPE histology of osteochondral samples

31.5 Spatial transcriptomics from osteochondral tissues

14% EDTA is used for the decalcification process of the osteochondral tissues.

14% EDTA solution: 280g EDTA (Sigma Cat# ED, *not di-sodium), 1.5L deionized water, 180mL Ammonium Hydroxide. Adjusted pH is 7.1.

Samples are decalcified on a rocker at RT for 6-7 days, with one solution changed on day 3. Once samples are decalcified, samples are inserted into cassettes and put onto the automatic tissue processor. Samples are then embedded, sectioned, and stained for Safranin O/Fast Green. When samples are used for Nanostring-GeoMx or 10X-Xenium, blocks are cut according to the standard protocols of the company.

32.0 List of Reagents and Supplies

32.1 Harvester PPE:

NoCry cut resistant gloves
Surgical mask with faceshield or surgical mask and safety goggles
Shoe covers
Surgical gown
Nitrile gloves
Sterile gloves

32.2 Harvesting station:

Absorbent pads
Sterilization wraps
Sterile surgical half drape
PBS
Sterile gauze

32.3 Photography equipment for macroscopic imaging:

Copy stand
Black cardstock paper for background
Glass to place samples on
Scale/ruler
2 lights attached to light arms angled at 45°angles
Powersource or rechargeable powerbank for lights
Nikon D5600
35mm f/1.8 AF-S DX lens
Polarizing filter

32.4 Tissue collection and processing:

15mL conical tubes
50mL conical tubes
5mL Eppendorf tubes (RNAse/DNAse free)
2mL Eppendorf tubes (RNAse/DNAse free)
1.5mL Eppendorf tubes (RNAse/DNAse free).
Plastic Scintillation Vials
2mL internal thread cryovials
4oz sample collection containers, non-sterile (Fisher 14-828-321)
8oz wide plastic containers (Fisher 14-955-115B)
10mL syringes

18G sterile needles
#11 and #21 scalpels
Straight razor blades
Cutting boards
Surgical scissors
Forceps
IMEB benchtop pathology saw
100mm and 150mm petri dishes
Tissue marking dye (CDI)
AllProtect
Zinc buffered formalin (Zfix – Anatech)
OCT
Standard and intermediate cryomolds
Standard and mega histology cassettes
KP Plus Markers/Pencil (for labeling cassettes)
Blue biopsy sponges (for synovium)
22% Formic Acid
14% EDTA
Liquid Nitrogen
Dry Ice
Wet Ice
Rocker or orbital shaker
4°C, -20°C, -80°C and LN storage