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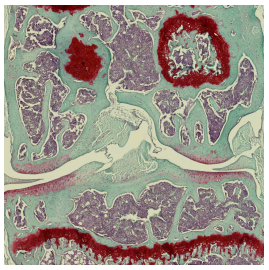
Knee joint histology for evaluation of osteoarthrits in mice



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We use this protocol and it's working

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Abstract

This protocol details how to extract and process mouse knee joints for the evaluation of osteoarthritis pathology in mice.

Materials

Equipment:
Scalpel blades (for cutting leg at hip joint) – best to use scalpel blade holder with disposable blades so you don't end up using lots of the plastic ones.
1 x forceps
1 x Black scissors for cutting skin and trimming muscle

Reagents for processing:
Pentobarbitone or similar anaesthetic for perfusion
1xPBS
10% neutral buffered formalin (Sigma, UK)
0.5 M EDTA pH 7.4 made up in PBS (Sigma, UK)
70% ETOH
90% ETOH

Reagents for staining:
0.05% Fast Green (FCF) Solution made up in distilled water (Merk F7252-5G)
1% Acetic Acid Solution made up in distilled water (Glacial Fisher A/0400/PB15)
0.1% Safranin O Solution (Sigma S2255-25G) made up in distilled water
Xylene (Histological grade Honeywell 534056)
Haematoxylin (Alfa Cesar A12431)
Eosin Y (Sigma E-6003)
95% ethyl alcohol (Absolute ethanol Sigma 32221)
DPX mounting medium (ThermoFisher LAMB/DPX)
Paraffin wax: Fibrowax VWR 361427G
Slides and cover slips

Mouse perfusion and dissection

1 Inject mouse with 200 mg/kg pentobarbitone.

2 Once mouse is deeply anaesthetised, perform a transcardial perfusion with 20ml PBS using a 25 ml syringe with a 27g needle (brown) attached.

3 Dissect the knee joint on the correct side (usually the right side for OA surgical models).

3.1 Remove skin from around leg using scissors and forceps.

3.2 Remove the entire leg by cutting with a scalpel blade near to the hip joint.

3.3 Using sharp scalpel blade or scissors remove as much of the muscle as possible from around the knee joint.

Take care not to damage or twist the joint itself.

3.4 Leave at least 15 mm of bone either side of the knee joint. It can be cut down more later once decalcified.

Fixation and tissue processing

4 Put joint into 25ml of 10% neutral buffered formalin and leave for 24 hours to fix on a slow rocking table.

5 Decalcify in 50mL 0.5 M EDTA pH 7.4 and leave to rock gently for 7-10 days.

This is usually enough time – you can check how decalcified joints are by cutting bone off long ends with sharp scalpel. Discard EDTA and add fresh EDTA if they are still crunchy.

6 After EDTA, trim the excess bone.

7 Rinse for three times in distilled water on the rocker (20 mins each).

8 Place in 70% ETOH and change the solution 3 times in 1 hour.

9 Place in 90% ETOH and hold at 4 degrees.

Processing, embedding and cutting:

10 Process to molten paraffin wax, using a standard procedure.
We use a Leica carousel processing machine (TP1020) and perform the following steps:

10.1 2 hours in 90% Ethanol

10.2 3 × 2 hours in 100% Ethanol

10.3 2 hours in Ethanol and Xylene (1:1 ratio)

10.4 3 × 2 hours in Xylene

10.5 2 × 2 hours in molten paraffin wax

11 Embed in paraffin wax.

11.1 Prepare mould of suitable depth and size with molten paraffin wax.

11.2 For coronal sectioning (to get medial and lateral tibio-femoral joints), place the anterior face of the knee joint down so that the patella tendon is positioned at the bottom of the metallic mould.

This step is critical – ensure the knee joint remains in position and does not fall onto its side.

12 Once block has cooled, cut coronal sections from the central compartment of the joint using a microtome (≥ three slides with 5 × 6µm sections; 66 µm section intervals).

See Blease et al, 2018 protocol [1] for more detailed information

Staining:

13 Deparaffinize sections with Xylene and hydrate with alcohol to distilled water.

14 Stain with hematoxylin solution for 10 minutes.

15 Wash in running tap water for 10 minutes.

16 Stain with Fast green solution for 5 minutes.

17 Blot slide rack on tissue to dislodge excess dye.

18 Rinse quickly with 1% acetic acid solution for no more than 10 –15 seconds.

19 Stain in 0.1% safranin O solution for 5 minutes.

20 Quickly, rinse briefly with x2 changes with distilled water.

21 Rinse briefly with 2x changes with 95% alcohol.

22 Use paper tissue to dislodge excess water/alcohol and then immediately place in oven preheated to 60C. Leave for 1 hour.

23 Place slide rack into mounting Xylene pot. Leave for 5 mins, then mount using DPX mounting medium.

Image aquisition

24 Image sections in brightfield mode at 10x using a microscope. A slide scanner (e.g. Axioscan 7, Zeiss) can be used to facilitate image acquisition.

Protocol references

1 Blease, A. *et al.* Studying Osteoarthritis Pathogenesis in Mice. *Curr Protoc Mouse Biol* **8**, e50, doi:10.1002/cpmo.50 (2018).