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## Tissue dissection, freezing and cell preparation

### Myometrial Group

#### 1.0 Purpose and scope.

Tissue biopsy samples will be collected from consented patients for research purposes.  
Fresh tissues may be subjected to enzyme digestion for cell isolation and culturing.  
Tissues may be placed directly into liquid nitrogen or may be cryoprotected and frozen in liquid nitrogen cooled isopentane.  
Fresh tissues may be dissected for functional experiments.

#### 2.0 COSHH / Health & Safety

Appropriate COSHH forms should be completed for the use of isopentane, risk assessments for the use of liquid nitrogen and also BIOCOSHH assessment of the risk of exposure to unfixed tissues.

##### **Hazard assessment**

Infectious: Hepatitis B, C, HIV (route of injury: needle injury/scalpel wound/exposed wounds).  
Isopentane - Highly flammable, has degreasing effect on the skin which may result in inflammation.

Ingestion causes nausea and vomiting and in large quantities may cause drowsiness, dizziness, euphoria, excitation, spasms and in certain circumstances, narcosis.

Liquid nitrogen must be used in a well ventilated area with oxygen level monitors present.

##### **Toxicity**

Isopentane - No evidence of carcinogenic properties or of mutagenic or teratogenic effects.

##### **Storage**

Isopentane - Flammable bin.

##### **PPE**

Eye protection, nitrile gloves, heavy duty insulated gloves for use with liquid nitrogen to protect skin from burns.

##### **Spillages**

Isopentane - Shut off all sources of ignition. Mop up spill with paper towel. Wash area with copious amounts of water. Dispose of contaminated materials in a sealed yellow clinical waste bag.

Liquid nitrogen – Evacuate area until oxygen levels restored.

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### **Waste disposal**

Isopentane – Small amounts of liquid (<10ml) can be left to evaporate in a fume hood. Larger volumes of liquid must be disposed of via an accredited disposal contractor. Store waste in a flammable bin prior to disposal.

### **Training**

Ensure that all staff are familiar with safe handling procedures and have read and understood the COSHH assessment.

## **3.0 Equipment / reagents**

Dewar flask  
Isopentane  
Metal freezing bath  
Liquid nitrogen  
Labelled Freezer bags/boxes  
Sterile Petri Dishes  
Collagenase/elastase/trypsin inhibitor buffer  
Dissecting dishes  
Dissecting instruments

## **4.0 References**

- 4.1 HTA**
- 4.2 University/Faculty Policy**
- 4.3 Royal Victoria Infirmary**

## **5.0 Procedure**

### **5.1 Dissecting tissue for functional experiments**

1. Tissue biopsies are placed in a dissecting dish and covered with a physiological salt solution.
2. Tissue is micro-dissected under a stereo microscope for use in various functional experiments.
3. Amend records accordingly.
4. Tissue debris is disposed of as per SOP: USC03b

### **5.2 Culturing Cells**

1. All tissue digestion is carried out inside a microbiological safety cabinet.
2. Tissue biopsies are rinsed in 1 X HBSS solution.
3. Tissue is cut up in sterile petri dishes with scissors and scalpel
4. Tissue is placed in collagenase digestion buffer and incubated at 37C to isolate cells for culture.
5. Amend records accordingly.
6. Tissue debris is disposed of as per SOP: USC03b

### 5.3 Freezing tissue

1. Pour a small amount of isopentane into a metal freezing bath.
2. Slowly lower the bath into a dewar containing liquid nitrogen. **DO NOT** submerge the beaker. Leave to freeze for several minutes.
3. Prepare the tissue blocks as required.
4. Remove the freezing bath from the liquid nitrogen and allow to thaw sufficiently so as to allow complete submersion of the tissue blocks. This can be speeded up by thawing a central well using a spatula. The isopentane should be at its melting point – ensure that there is still some frozen isopentane in the bath.
5. Freeze the tissue by plunging into the isopentane for a few seconds while agitating gently.
6. Blot off any excess isopentane before placing samples into storage containers.
7. Remove the freezing bath to a fume hood to allow the isopentane to evaporate.
8. Amend records accordingly.
9. Tissue debris is disposed of as per SOP: USC03b

### 6.0 Site Specific Details

Personnel: Newcastle Uteroplacental Tissue Bank Users,

Location: G205, 2nd Floor, East Wing, ICfL

Induction: All are provided with a written protocol with safety details. Demonstration is given with continuing supervision as necessary.