扫描电镜实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
临界点干燥仪	Quorum	K850
离子溅射仪	HITACHI	MC1000
扫描电子显微镜	HITACHI	SU8100

2、 主要实验试剂

试剂	厂家	货号
电镜固定液	Wanwu	G1102
无水乙醇	国药集团化学试剂有限公司	100092183
乙酸异戊酯	国药集团化学试剂有限公司	10003128
PBS	Wanwu	G0002
锇酸	Ted Pella Inc	

二、扫描电镜制样步骤

1、**取材固定：**新鲜组织确定取材部位，尽量减小牵拉、挫伤与挤压等机械损伤，1-3min内取样，组织块面积不超过 3mm^2，用 PBS 轻轻漂洗将样本表面的血污，毛发等去掉，保护好需要扫描的面并做好标记（如在对面进行剪角处理）。迅速投入电镜固定液室温固定 2h，再转移至 4°保存。

贴壁细胞：贴壁于盖玻片的细胞培养处理完成后弃培养基，用 PBS 轻轻漂洗后，弃 PBS 加电镜固定液室温固定 2h，再转移至 4°保存。注意保护好扫描面避免剧烈震荡细胞脱落。

2、**后固定：**固定好的样品经 0.1M 磷酸缓冲液 PB（PH7.4）漂洗 3 次，每次 15min。0.1M 磷酸缓冲液 PB（PH7.4）配制 1%锇酸室温避光固定 1-2h。0.1M 磷酸缓冲液 PB（PH7.4）漂洗 3 次，每次 15min。

3、**脱水：**组织依次入 30%-50%-70%-80%-90%-95%-100%-100%酒精每次 15min,乙酸异戊酯 15min。

4、**干燥：**将样本放入临界点干燥仪内进行干燥。

（干燥的样品及无机材料不需要以上步骤）

5、**样本导电处理：**将样本紧贴于导电碳膜双面胶上放入离子溅射仪样品台上进行喷金 30s 左右。

6、扫描电子显微镜下观察采图。

Scanning Electron Microscopy Report

1. Apparatus and Reagents

1.1 Major Apparatus

Name	Producer	Model
Critical Point Dryer	Quorum	K850
Lon Sputtering Apparatus	HITACHI	MC1000
Scanning Electron Microscope	HITACHI	SU8100

1.2 Major Reagents

Name	Producer	Code
Fixative for TEM	Wanwu	G1102
Ethanol	Sinopharm Group Chemical Reagent Co. LTD	100092183
Isoamyl acetate	Sinopharm Group Chemical Reagent Co. LTD	10003128
PBS	Wanwu	G0002
OsO_4	Ted Pella Inc	

2. Procedure

2.1 **Harvest tissue block and fixation**: Targeted fresh tissues should be selected to minimize mechanical damage such as pulling, contusion and extrusion. Use a sharp blade to cut and harvest fresh tissue blocks quickly within 1-3 minutes. The area of tissue block should be no more than 3 mm^2. Wash tissues with PBS gently to remove the blood and hair, etc. Label the target side of tissue (the side you want to observe) by any way, such as making cuts on the opposite side. Make sure to protect tissue blocks, especially the target side, from mechanical damage such as forceps extrusion. The washed tissue blocks are immediately fixed by electron microscopy fixative for 2 hours at room temperature, then transferred into 4°C for preservation and transportation.

For adherent cell: Seed cells on a sterile cover glass in a petri dish. Remove the culture medium, then wash slide gently with PBS, followed by adding electron microscopy fixative into petri dish. After fixing for 2 hours at room temperature, transfer the petri dish to 4°C for preservation and transport. Note to avoid sever shock which may result in the cells dropping off from cover glass.

2.2 **Post-fix:** Wash tissue blocks with 0.1 M PB (pH 7.4) for 3 times, 15 min each. Then transfer tissue blocks into 1% OsO_4 in 0.1 M PB (pH 7.4) for 1-2 h at room temperature. After that, wash tissue blocks in 0.1M PB (pH 7.4) for 3 times, 15 min each.

2.3 Dehydrate as followed:

30% ethanol for 15 min;

50% ethanol for 15 min;

70% ethanol for 15 min;

80% ethanol for 15 min;

90% ethanol for 15 min;

95% ethanol for 15 min;

Two changes of 100% ethanol for 15 min;

Finally, isoamyl acetate for 15 min.

2.4 Drying: Dry samples with Critical Point Dryer.

Note: Dry samples and inorganic materials should ignore all the steps above and do the following step directly.

2.5 Conductive metal coating: Specimens are attached to metallic stubs using carbon stickers and sputter-coated with gold for 30s.

2.6 Observe and take images with scanning electron microscope.