动物组织透射电镜实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
超薄切片机	Leica	Leica UC7
钻石切片刀	Daitome	Ultra 45°
透射电子显微镜	HITACHI	HT7800/HT7700
150 目方华膜铜网		

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
丙酮	国药集团化学试剂有限公司	10000418
812 包埋剂	SPI	90529-77-4
锇酸	Ted Pella Inc	

二、透射电镜制片步骤

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，取样组织 1mm³ 大小。取材前可提前准备装有电镜固定液的培养皿，将小组织块离体取下后立即投入培养皿内，用手术刀在培养皿的固定液中进行切割成 1mm³ 的小组织块。再将切割好的小组织块转移至装有新的电镜固定液的 EP 管内继续固定，4℃固定保存及运输。0.1M 磷酸缓冲液 PB（PH7.4）漂洗 3 次，每次 15min。

2、**后固定：**0.1M 磷酸缓冲液 PB（PH7.4）配制的 1%锇酸避光室温固定 2h。0.1M 磷酸缓冲液 PB（PH7.4）漂洗 3 次，每次 15min。

3、 **室温脱水：**组织依次入 30%-50%-70%-80%-95%-100%-100%酒精上行脱水每次 20min，100%丙酮两次，每次 15min。

4、 **渗透包埋：**丙酮：812 包埋剂=1：1 37℃ 2-4h， 丙酮：812 包埋剂=1：2 37℃渗透过夜，纯 812 包埋剂 37℃ 5-8h。将纯 812 包埋剂倒入包埋板，将样品插入包埋板后 37℃烤箱过夜。

5、 **聚合：**包埋板放于 60℃烤箱聚合 48h，取出树脂块备用。

6、 **超薄切片：**树脂块于超薄切片机 60-80nm 超薄切片，150 目方华膜铜网捞片。

7、 **染色：**铜网于 2%醋酸铀饱和酒精溶液避光染色 8min；70%酒精清洗 3 次；超纯水清洗 3 次；2.6%枸橼酸铅溶液避二氧化碳染色 8min；超纯水清洗 3 次，滤纸稍吸干。铜网切片放入铜网盒内室温干燥过夜。

8、 透射电子显微镜下观察，采集图像分析。

TEM staining report for animal tissue specimen

1 Apparatus and reagents

1.1 Major apparatus

Name	Producer	Model
Ultra microtome	Leica	Leica UC7
Diamond slicer	Daitome	Ultra 45°
Transmission Electron Microscope	HITACHI	HT7800/HT7700
150 meshes cuprum grids with formvar film		

1.2 Major reagents

Name	Producer	Code
Fixative for TEM	Wanwu	G1102
Ethanol	Sinaopharm Group Chemical Reagent Co. LTD	100092183
Aceton	Sinaopharm Group Chemical Reagent Co. LTD	10000418
EMBed 812	SPI	90529-77-4
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 size of tissue block should be no more than 1 mm^3. Before sampling, petri dishes with fixative for TEM should be prepared in advance, small tissue blocks could be removed from animal body and immediately put into petri dishes, and then cut into small size of 1 mm^3 in the fixative. The 1mm^3 tissue blocks were transferred into an EP tube with fresh TEM fixative for further fixation, which was fixed at 4℃ for preservation and transportation. And then wash the tissues using 0.1 M PB (pH 7.4) for 3 times, 15 min each.

2.2 **Post-fix:** Tissues avoid light post fixed with 1% OsO_4 in 0.1 M PB (pH 7.4) for 2 h at room

temperature. After remove OsO_4, the tissues are rinsed in 0.1 M PB (pH 7.4) for 3 times, 15 min each.

2.3 Dehydrate at room temperature as followed:

30% ethanol for 20 min;

50% ethanol for 20 min;

70% ethanol for 20 min;

80% ethanol for 20 min;

95% ethanol for 20 min;

Two changes of 100% ethanol for 20 min;

Finally two changes of acetone for 15 min.

2.4 Resin penetration and embedding as followed:

Acetone：EMBed 812=1:1for 2-4 h at 37℃;

Acetone：EMBed 812=1:2 overnight at 37℃;

pure EMBed 812 for 5-8 h at 37℃;

Pour the pure EMBed 812 into the embedding models and insert the tissues into the pure EMBed 812, and then keep in 37℃ oven overnight.

2.5 Polymerization: The embedding models with resin and samples were moved into 65℃ oven to polymerize for more than 48h. And then the resin blocks were taken out from the embedding models for standby application at room temperature.

2.6 Ultrathin section: The resin blocks were cut to 60-80nm thin on the ultra microtome, and the tissues were fished out onto the 150 meshes cuprum grids with formvar film.

2.7 Staining: 2% uranium acetate saturated alcohol solution avoid light staining for 8 min, rinsed in 70% ethanol for 3 times and then rinsed in ultra pure water for 3 times. 2.6% Lead citrate avoid CO_2 staining for 8 min, and then rinsed with ultra pure water for 3 times. After dried by the filer paper, the cuprum grids were put into the grids board and dried overnight at room temperature.

2.8 Observation and images capture: The cuprum grids are observed under TEM and take images.