细胞样本透射电镜实验报告

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
200 目方华膜铜网	中镜科仪	BZ11262a
离心机	湖南湘仪实验室仪器开发有限公司	TG16-W

2、 主要实验试剂

试剂	厂家	货号
电镜固定液	biossci	BP0130
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-Chem	02660
锇酸	Ted Pella Inc	18456
醋酸双氧铀	SPI-Chem	02624-AB
柠檬酸铅	emsdiasum	17800
琼脂糖	Solarbio	A8201

二、透射电镜制片步骤

1、取材固定：离心收集细胞或细菌沉淀，4°C固定保存及运输。

2、琼脂预包埋：细胞悬液用离心机1000-2000rpm离心5-10min(根据实际情况适当增加速度)，弃上清加入 0.1M 磷酸缓冲液 PB（PH7.4），混匀漂洗 5min 后再1000-2000rpm离心5-10min(根据实际情况适当增加速度)，重复洗涤 3 次。弃去上清，细胞沉淀中提前加热溶解制备 1%琼脂糖溶液，稍冷却后加入 EP 管内，在琼脂糖凝固之前将细胞沉淀用牙签挑起悬浮包裹于琼脂糖内，细胞丰富区域切成1mm^3大小。

3、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。

4、 室温脱水：组织依次入 30%-50%-70%-80%-95%-100%-100%酒精上行脱水每次 10min，100%丙酮两次，每次 10min。

5、 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。

6、 聚合：包埋板放于 60℃烤箱聚合 48h，取出树脂块备用。

7、 超薄切片：树脂块于超薄切片机 70nm 超薄切片，200 目方华膜铜网捞片。

8、 染色：铜网于 2%醋酸铀饱和酒精溶液避光染色 10min；超纯水清洗3 次；枸橼酸铅溶液避二氧化碳染色 5-10min；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。

9、透射电子显微镜下观察

TEM staining report for cells and bacteria

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
2000 meshes cuprum grids with formvar film	emcn	BZ11262a

1.2 Major reagents

Name	Producer	Code
Fixative for TEM	biossci	BP0130
Ethanol	Sinaopharm Group Chemical Reagent Co. LTD	100092183
Aceton	Sinaopharm Group Chemical Reagent Co. LTD	10000418
EMBed 812	SPI	02660-AB
OsO4	Ted Pella Inc	02602-AB
uranyl acetate	Ted Pella Inc	02624-AB
Agarose	Solarbio	A8201
Lead Citrate	emsdiasum	17800

2 Procedure

2.1 **Harvest samples and fixation**: Collect cells or bacteria precipitation after centrifuge. The TEM fixative was added to the tube and let the precipitation re-suspended in the fixative, and then fixed at 4℃ for preservation and transportation.

2.2 **Agarose pre-embedding**: The fixed cells and bacteria were centrifuged. The 0.1 M PB (pH 7.4) was added into the tube after supernatant was discarded, and then the precipitation was re-suspended and washed in PB for 3min. This washing step was repeated for 3 times. The 1% agarose solution was prepared by heating and dissolving in advance. After being cooled, the agarose solution was added into the EP tube. Before agarose

solidification, the precipitation was suspended with toothpick and wrapped in the agarose.The size of cells precipitation should be no more than 1 mm3

2.3 Post-fix: Agarose blocks with samples 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.

2.1 Dehydrate at room temperature as followed:

30% ethanol for 10 min;

50% ethanol for 10min;

70% ethanol for 10min;

80% ethanol for 10min;

95% ethanol for 10min;

Two changes of 100% ethanol for 10 min;

Finally two changes of acetone for 10min.

2.4 Resin penetration and embedding as followed:

Acetone：EMBed 812= 1:1for 2-4h at37℃

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 60℃ 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 200 meshes cuprum grids with formvar film.

2.7 Staining: 2% uranium acetate saturated alcohol solution avoid light staining for 10 min, then rinsed in ultra pure water for 3 times. Lead citrate avoid CO_2 staining for 10 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.