



骨和植物-MMA 包埋实验报告

一、实验器材及试剂

1、实验器材

名称	厂家	型号
硬组织切片机	Leica	HistoCore AUTOCUT
钨钢切片刀	Leica	TC-65
生物组织摊片机	KEDEE	KD-P

2、主要实验试剂

试剂	厂家	货号
通用型组织固定液	Servicebio	G1101
无水乙醇	国药集团化学试剂有限公司	100092683
二甲苯	国药集团化学试剂有限公司	10023418
乙二醇乙醚乙酸酯	麦克林	E808814
甲基丙烯酸甲酯	麦克林	M813511
邻苯二甲酸二丁酯	国药	10008518
过氧化苯甲酰	麦克林	B802244

二、硬组织切片制片步骤

- 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，4%PFA 固定骨组织，70%FAA 固定植物，固定至少 48 h。注意，组织取材厚度大约 5 mm 左右，最大长度 3 cm，骨组织尽量将表面的肌肉剃干净，以免后期影响结果观察。
- 脱水：**组织依次入 60%-70%-80%-90%-95%酒精脱水，每梯度 24 h-72 h，100%I 12 h-24 h，100%II 12 h-24 h，二甲苯 8-24 h。
- 浸塑：**浸塑液I 4℃浸塑 24-72 h，浸塑液II 4℃浸塑 24 h-72 h，浸塑液III 4℃浸塑 24 h-72 h。
- 包埋：**将组织放入包埋瓶，加适量包埋液，抽真空 5 h，37℃水浴聚合。
- 切片：**硬组织切片机切片 10 μ m，60℃过夜烤片。



MMA Embedding

1. Experimental equipment and reagent

1.1 Equipment

Brand name	Manufactures	Model
Hard tissue slicer	Leica	HistoCore AUTOCUT
Disposable blades	Leica	TC-65
Water Bath	KEDEE	KD-P

1.2 Reagent

Reagent	Manufactures	Cat
4% PFA Fix Solution	Servicebio	G1101
70% FAA Fix Solution	Servicebio	G1103
Ethanol absolute	Sinopharm Chemical Reagent Co.,Ltd	100092683
Xylenes	Sinopharm Chemical Reagent Co.,Ltd	100023418
Methyl methacrylate	Macklin	M813511
Dibutyl phthalate	Sinopharm Chemical Reagent Co.,Ltd	10008518
Dibenzoyl peroxide	Macklin	B802244

2. Procedure for sectioning hard tissue

2.1. Draw materials

Fresh tissue was used to determine the sampling site and minimize mechanical damage such as pulling, contusing and squeezing. 4%PFA fixed bone tissue and 70%FAA fixed plants for at least 48 hours. It should be note that the tissue sampling thickness is about 5 mm and the maximum length is 3 cm. The bone tissue should try to shave the surface muscles, for fear that affect the observation of the results.

2.2. Dehydration

The tissue is then dehydrated in 60% - 70% - 80% - 90% - 95% alcohol. Each step takes anywhere for 24 to 72 hours. Next, absolute ethyl alcohol is used twice to dehydrate and 12 h to 24 h each time. The same is true for xylene.



2.3. MMA infiltration

Put tissue into three cylinders of permeates penetrating fluid at 4°C for 24 to 72 hours in turn.

2.4. MMA embedding

The tissue was put into the embedding bottle, and an appropriate amount of embedding agent was added. Then vacuumed for 5 h and polymerized in a 37°C water bath.

2.5. Sectioning

Routine sectioning about 10 μm by hard tissue slicer and place sections at 60°C oven overnight.