

Supplementary article data

A single topical dose of erythropoietin applied on a collagen carrier enhances calvarial bone healing in pigs

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MMA IV embedding technique for embedding of bone samples

This protocol describes a methyl methacrylate (MMA) embedding method. It is the standard embedding procedure used for bone samples at the Orthopaedic Research Laboratory, Aarhus University Hospital, Denmark.

The MMA IV technique is used for the following purposes: bone histomorphometry, histochemistry, and immunohistochemistry. It is a modification of: Erben RG. Embedding of bone samples in methylmethacrylate: an improved method suitable for bone histomorphometry, histochemistry, and immunohistochemistry. *J Histochem Cytochem* 1997; 45(2):307–13.

Ideally, the entire procedure should be performed cold and under vacuum.

Stepwise dehydration, infiltration, and embedding

1. Dehydration

70% ethanol.

96% ethanol.

96% ethanol.

100% 2-propanol.

100% 2-propanol.

Xylene.

Xylene.

2. Infiltration in MMA IV mixture

Mix the following reagents:

80 mL MMA (cat. no. 8.00590; Merck KGaA, Darmstadt, Germany).

20 mL Grindsted Soft-N-Safe (cat. no. 175540; DuPont Danisco, Grindsted, Denmark).

1 mL polyethylene glycol 400 (cat.no. 8.07485; Merck KGaA Darmstadt, Germany).

0.8 g benzoyl peroxide (cat. no. 8.01641; Merck KGaA Darmstadt, Germany).

NB: Weigh out 1 g of benzoyl peroxide and air-dry for 4–6 h, before using 0.8 g.

Stir for at least 1 h and store at 4°C until use.

3. Embedding procedure:

100 mL MMA IV mixture (at 4°C).

300 µL N,N-dimethyl-p-toluidine (cat. no. 8.22040; Merck KGaA Darmstadt, Germany) which accelerates the embedding.

Stir for a few minutes while supplying with gaseous N₂

Place the infiltrated tissue in an airtight container.

Fill the container with embedding mixture.

Supply with gaseous N₂ for a short time and seal the container.

Store at –20°C for 4–7 days until hardened.

NB: Because an exothermic reaction occurs, immediate placement in the freezer is mandatory.