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Musculoskeletal Cell Core

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Isolation of Primary Osteoblasts from Calvaria

Materials:

- Collagenase type I (Worthington –CLS1)
- Collagenase type II (Worthington –CLS2)
- HEPES solution (1M pH 7.4)
- Bovine Serum Albumin (powder or 10% stock solution)
- aMEM (Gibco 12571-063)
- Complete medium (aMEM + 10% Fetal Bovine Serum (Gibco 26140-079 lot#1883391) + 1% Pen-Strep or Antimitotic/Antibiotic – GIBCO 15240-062)

For digestion:

A) Collagenase solution: 2.5 mg/ml collagenase1+collagenase 2 (ratio 1:3), 2.5 mM CaCl₂ (solution should be made in ddH₂O and filtered). Keep on ice

B) Medium: alpha MEM + 0.1 % BSA + 25 mM HEPES pH 7.4. medium should be at 37C

C) EDTA solution: 5mM EDTA in PBS + 0.1 % BSA (filtered)

D) Medium: Alpha MEM + 10%FBS+1%PS

Procedure:

Keep pups on ice.

Quickly dip in EtOH, cut head, remove skin and isolate calvarial bones (parietal only)

Put the bone into serum free ice cold medium and keep them on ice.

At the end of all isolation prepare collagenase solution; Mix medium (B)+ collagenase (A) (2.5:1 ratio) to obtain final collagenase solution of 1mg/ml.

Put calvarial bone into wells (if single calvaria =1 bone in 1 12well-plate, if combined 3-5 bones/1 6well) 12 or 6 well plate (depends on experiment) and add 1 ml (for 12-well) or 2 ml (for 6 wellplate) of collagenase solution (medium plus collagenase). Incubate at 37 C (plate warmer) on a shaker (~ 90 rpm/min = setting on 5)

Digestion 1= 15 min collagenase solution.

Digestion 2= 20 min collagenase solution.

Digestions 1 +2 are discarded



Digestion 3-4 and 6 = 15 min collagenase. Collect medium and add to a 15 ml tube containing 5 ml of complete medium a-MEM+10%FBS+PS (the FBS will inactivate the collagenase). Keep on ice. Wash the calvaria with 1 ml of collagenase solution and collect the wash in the same tube. Collect together all 4 digestions. Spin, aspirate medium, resuspend in fresh and plate (Osteoblast enriched population)

Digestion 5= 15 min EDTA. Wash once with EDTA solution. Collect cells

For osteocytes enriched population:

Digestion 5, 7= 15 min EDTA digestion. No wash needed. Just collect cells.

Digestion 8= 15 min collagenase. Same as for digestion 3. Collect together with digestion 7. Spin and plate (Osteocyte enriched population)