Creighton
UNIVERSITY

Preparation of bone fragments for the transfer of the hematopoietic microenvironment

(unknown)

Equipment:

Beaker
Stir plate & stir bar
74-120 µm sieve

Reagents:

Distilled water
Absolulte Ethanol
Ether
1 N HCl

Method:

1. Remove ends of femur and flush out marrow.
2. Soak femur in distilled water for 2 hours with constant stirring, changing the water every 40 minutes.
3. Place the femur in 100% ethanol for 1 hour with constant stirring.
4. Place the femur in ether for 30 minutes with constant stirring.
5. Using a mortar and pestle, crush the bone.
6. Pass the crushed bone fragments through a 74-120 µm sieve to remove chunks.
7. Treat the crushed bone with 0.5 N HCl for 6 hours.
8. Treat the crushed bone with distilled water for 2 hours with constant stirring, changing the water every 40 minutes.
9. Wash the bone in 100% ethanol for 1 hour with constant stirring.
10. Place the crushed bone in ether for 30 minutes with constant stirring.
11. Air-dry the crushed bone.
12. For culturing, place approximately 10 mg of bone in each tube with culture media.