Human subchondral osteoblasts cell culture

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Abstract

Method of isolation and culture of osteoblast coming from human subchondral bone of the knee

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Materials

- HEPES H6147 by Sigma Aldrich
- Trypsin EDTA 25-051-CI. by Gibco - Thermo Fischer
- 200 mM L-Glutamine G7513 by Sigma
- DMEM 4.5 g/L glucose BE12-614F by Lonza
- Collagenase IA C9891 by Sigma Aldrich
- ✓ Fetal Bovine Serum S1810 by Contributed by users
- Penicilin Streptomycin DE17-602E by Lonza
- PBS 1x BE17-516F by Lonza

Protocol

Subchondral bone dissection

Step 1.

Tibial subchondral bone plates were collected.

After careful elimination of trabecular bone and articular cartilage, subchondral bone was dissected to separate sclerotic from non-sclerotic zones. Non-sclerotic and sclerotic bone zones were identified by a marked difference in thickness. We considered sclerotic bone to be only that from the subchondral areas of bone with a thickness >2 mm and being either denuded or overlaid by fibrillated cartilage. We considered nonsclerotic bone to be only that from the subchondral areas of bone with a maximal thickness of 1 mm. Intermediate zones of the subchondral bone plate were discarded.

The plates were then cut into small fragments of approximately 2 mm^3 with a bone rongeur, washed with DMEM and then submitted to enzymatic digestions.

Subchondral bone digestion

Step 2.

Small pieces of bone (2 mm^3) were sequentially incubated with 1 mg/ml clostridial collagenase IA (Sigma-Aldrich) for 35 and 240 min successively (2 g of bone in 20 ml of enzymatic solution).

Subchondral osteoblast culture

Step 3.

The digested bone pieces were extensively rinsed in DMEM, placed into T-75 flasks (0.5 to 1 g of bone per flask for not sclerotic and 1.5 to 2 g of bone for sclerotic) and cultured in DMEM supplemented with 15% fetal bovine serum (FBS), 10 mM HEPES, 100 U/ml penicillin and 100 µg/ml streptomycin, until osteoblasts migrated out of bone explants (one week). Media was replaced twice a week. At this point, the medium was replaced with fresh media containing 10% FBS, 10 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM glutamine. At confluence, cells were collected by trypsinization, seeded (20,000 cells/cm^2) in 6, 12 or 24-well plates (NUNC Nunclon plates) and grown for three days in DMEM containing 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mM HEPES and 2 mM glutamine.

Subchondral osteoblast culture

Step 4.