Development of primary cell cultures from calcified tissues of marine fish

1. Fish is anesthetized with 2-phenoxyethanol diluted 1:10000 in seawater then sacrificed by decapitation. Wash it quickly with bleach diluted 1:100 in water then with 70% ethanol, to remove most surface contaminants.
2. Collect calcified tissues using sterile instruments and place them in PBS supplemented with 5% antibiotics and 1% fungizone. Clean them from adherent tissues using a scalpel and a tooth brush.
3. Mince tissues manually to small fragments (approx. 1 mm^3) using sterile instruments and wash them 5 times with PBS.
4. Fragments are digested with collagenase for 16-24 h at room temperature (approx. 22-24ºC) and with agitation.
5. Remove digestion solution and wash fragments 3 times with serum-free medium supplemented with 5% antibiotics and 1% fungizone.
6. Place fragments into a well of a 12-well plate containing 500 µl of medium supplemented with 15% FBS, 1% antibiotics and 0.2% fungizone. Incubate them at appropriate temperature (ranging from 18 to 33ºC depending on fish species).

   Factors essential to bone cell growth will diffuse from bone fragments throughout explants culture. It is important to seed fragments at high density for the successful development of primary cultures of bone cells.
7. Cells are allowed to migrate from fragments and attach to the plate for approximately 2-4 weeks with medium renewed twice a week. Conditioned medium (i.e. medium that has already been partially used by cells) is used to complement fresh medium (up to 50%).
8. Collect cells from confluent cultures by trypsinization (see below) and seed them into a 6-well plate with 2 ml of medium per well. When cultures are confluent again, transfer them into a 10-cm plate with 8 ml of medium. Complement fresh medium with conditioned medium.

   Trypsinization: Remove culture medium, wash cell layer with PBS and add trypsin-EDTA solution (1 ml per 10-cm dish). Monitor cell detachment microscopically and stop trypsin action by adding fresh medium supplemented with FBS.
9. Cell cultures are then routinely sub-cultured (1:2) by trypsinization and cryopreserved at appropriate passages (see protocol #2).

   Attention: Growth conditions (e.g. temperature, culture medium and serum percentage) should be optimized for each primary cell culture. Ideally, antibiotics and antimycotics should be removed after several passages and presence of mycoplasma should be tested regularly.

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Fish: Healthy juvenile(s) of marine fish bred in aquaculture conditions.

Apparatus: Cell incubators; a class II biological safety cabinet.

Cell culture medium: Dulbecco's modified Eagle medium (DMEM); Leibovitz's medium (L15).

Medium supplements: Fetal bovine serum (FBS); L-glutamine, antibiotics (penicillin & streptomycin); antimycotic (fungizone).

Solutions: phosphate-buffered saline solution (PBS: 137 mM NaCl, 2.7 mM KCl, 15.8 mM Na_2HPO_4, 1.23 mM KH_2PO_4; pH 7.4); digestion solution (0.125% collagenase in serum-free medium); trypsin-EDTA solution (1.1 mM EDTA, 0.2% trypsin in PBS).

Plasticware: 12-well, 6-well and 10-cm cell culture dishes and serologic pipettes.

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

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