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Original Article

A practical way to prepare primer human chondrocyte culture

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ABSTRACT

Biological cartilage repair is one of the most important targets for orthopedic surgeons currently. For this purpose, it is mandatory to know how to prepare a chondrocyte culture. In this study, our purpose was to introduce a method enabling orthopedic surgeons to practice their knowledge and skills on molecular experimental setup at cellular level, based on our experiences from previous pilot studies. Thus, we believe it will encourage orthopedic surgeons.

1. Introduction

Cartilage repair with cell therapies is a rapid developing area in regenerative medicine.[1] Recent developments in tissue engineering, pharmaceutical technology, and pharmacomolecular technology enabled researchers to use cell-based experimental methods.[3,4] Besides, cell therapies are being used to repair cartilage defects; gene therapy for an affected gene with RNA interference (siRNA/miRNA) is also used in hereditary diseases, such as Marfan syndrome, osteogenesis imperfecta, and chondrosarcoma.[5,6] In addition, cell culture methods are also currently used in biocompatibility studies of orthopedic materials, and osteo/chondro toxicity tests of pharmacological agents.

In order to use cells as an effective therapeutic for cartilage repair, they must survive and differentiate to the desired cell when they are implanted in a patient. Even if cell therapy applications have limited use in surgery at present, it could be foreseen that in a few years these studies would play an important role in many fields, including orthopedics. Therefore, it is necessary that an orthopedic surgeon is involved in these researches. For this reason, whether a researcher or a surgeon applying the treatment; an orthopedist should understand the methods to obtain and cultivating process of cells could be used in therapy.[2]

In this study, our purpose was to introduce a method enabling orthopedic surgeons to practice their knowledge and skills on molecular experimental setup, cell isolation from tissues, culture techniques, and characterization, with brief notes, based on our experiences from previous pilot studies.

2. Materials and methods

2.1. Minimum requirements for a cell culture laboratory

The first requirement, and perhaps the most important point for all cell culture laboratories, is ensuring aseptic conditions in the laboratory. In order to set up an experiment in vitro, there are some necessary equipments: an incubator which could be adjusted to 5% CO_2 and 37 °C, stereo and inverted microscope, class II biosecurity laminar airflow cabinet, a centrifuge, pH meter, vortex, heated magnetic stirrer, precision balance, sterilization system, distilled water, heated water bucket, refrigerator that could be adjusted at +4 °C, a deepfreezer that could be adjusted at −20 °C, cell counter or a Thoma Lam/Neubauer chamber, micropipettes ranging between 1 and 1000 μl, and sterile pipette tips. In addition to these equipments, necessary supplies like 5, 10, 25, and 50 mL disposable pipettes, 60–100 mm Petri dishes, glass Pasteur pipettes, autoclave resistant metal boxes, 15 and 50 mL conical centrifuge tubes, cell culture flasks, and 6-, 12-, 24- and 96-well plates are necessary. Syringe filters with 0.2 μm pore size are useful to sterilize low volume solutions.

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2.2. Cell culture mediums and solutions

Insulin, transferrin, and selenious acid containing premix solution (ITS), RPMI-1640 DMEM, inactivated fetal bovine serum (FBS), penicillin–streptomycin (PS), Amphotericin B, and L-glutamin are necessary to prepare a complete culture medium. Trypsin-2-[2-[bis(carboxymethyl)amino]ethyl-(carboxymethyl)amino]acetic acid (EDTA), phosphate buffered saline (PBS), dimethyl sulfoxide (DMSO), and collagenase type-II enzymes are other supplements to perform primary culture.

Eagle first described the basic amino acid combinations for cell cultures in 1955 and called it "minimum Eagle's medium (MEM)". This medium is still in use with some modifications. DMEM is one of these modified mediums used especially for solid tissue cultures. RPMI-1640 contains glucose, essential for the cell nutrition, appropriate osmolarity and pH for cell viability, necessary amino acids, and vitamins for the functionality. However, it is not sufficient for the development of chondrocyte and other cells alone.[4,7] For this reason, FBS and ITS should also be added to RPMI-1640 or DMEM medium in order to proliferate healthy, high confluent cells in chondrocyte cultures.[7,8] FBS contains the essential extracellular matrix proteins for cellular adhesion and provides relevant hormones, enzymes, and growth factors for cellular proliferation. Serum proportion may differ according to cell type and applications. In standard chondrocyte cultures, 10% serum ratio is usually sufficient. L-glutamin is usually supplied with RPMI-1640 and DMEM but it is preferable to add L-glutamin to complete medium. Thus this essential amino acid preserves as carbon source. For chondrocyte primary culture, we use 1 mL L-glutamin (from 200 mM stock solution) for 100 mL medium.

Penicillin–streptomycin (PS) and Amphotericin B are used in order to prevent contamination. 1 mL of penicillin–streptomycin (10,000 U/mL) and 2.50 μg/mL of Amphotericin B are sufficient for complete medium and also for transfer medium. PS concentration could be increased in transfer medium up to 5%, but it should be noted that antibiotics have antiproliferative effects on human tissue also.[4,7,8]

ITS will stimulate cell proliferation while decreasing substantially the serum requirements for culture of many cell types. Basal media supplemented with support proliferation of chondrocyte cultures. We used a commercial ITS solution containing 5 mg/ml insulin, 5 mg/ml transferrin, and 5 μg/mL selenious acid; 5 mL of this solution is sufficient for 5 l of medium.

Collagenase type II enzyme is used to digest osteochondral tissue and obtain chondrocytes detached from extracellular matrix. Trypsin–EDTA is another enzyme used in cell culture protocols, this time for detaching chondrocytes from culture dish to passage.

PBS, a saline solution that balances intra- and extracellular osmotic pressure, supports cellular metabolism through its inorganic salt and water content. Almost every washing step can be performed in PBS.

2.3. Preparation of optimal cell culture content for chondrocytes

Transfer medium prepared with 100 mL RPMI-1640 or DMEM contained 5 mL of penicillin–streptomycin (10,000 U/mL) and 2.50 μg/mL Amphotericin B. Complete medium prepared with 100 mL RPMI-1640 or DMEM contains 1 mL of penicillin–streptomycin (10,000 U/mL) and 2.50 μg/mL Amphotericin B, 1 mL L-glutamin (200 mM), 1 mL ITS, and 10% FBS.

2.4. Surgical harvesting and transfer of osteochondral tissue

Tissues from lateral and medial femoral condyles, and tibial plateau, are routinely removed during total knee arthroplasty (Fig. 1). After surgery, leftover osteochondral tissue is immediately transferred to cell culture laboratory in transfer medium under sterile conditions. Keeping tissue at 4 °C with ice packs prevents tissue from cell death. Attention to aseptic conditions, keeping tissue in cold, and immediate transfer are some of the key points for obtaining healthy primary cultures.

2.5. Preparation of primary chondrocyte cultures

Local ethics committee approval should be ready for the relevant animal or human tissue to be studied. If a human tissue is to be studied, an informed consent form is also necessary in addition to local ethics committee approval.

A common method to obtain single cell suspensions from primary tissue is enzymatic digestion. Exposing the cells to collagenase enzyme preserves maximum viability. The following procedures disaggregate whole tissue to obtain a high yield of viable cells. Note that all the following procedures are performed in a laminar flow cabinet.

Resected tissues from human or animal osteochondral tissue were transferred to cell culture laboratory and transferred to a Petri dish and smashed into small pieces, approximately 0.25 cm^3, either with scalpel or rongeur. To prevent from drying, the tissue is always kept in transfer medium or PBS. The tissue is transferred to 50 mL conical tubes and centrifuged at 1200 rpm for 10 min. The supernatant is discarded. To perform enzymatic digestion,

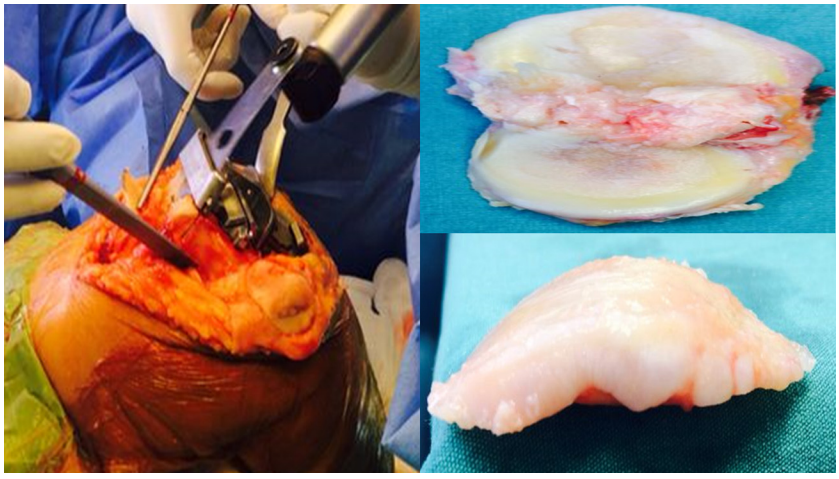

Fig. 1. Surgically excised osteochondral tissues.