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Cell response in mixtures of surfactant-culture medium—Towards a systemic approach to cell-based treatments for focal osteoarthritis

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ABSTRACT

Osteoarthritis (OA), the most common form of arthritis is a degenerative joint disease, which causes severe long-term pain and physical disability. It is becoming more important to improve diagnosis and understanding of the disease process and subsequently develop new intervention to delay or even reverse the disease progress. Our study was designed to combine two relatively novel treatment techniques, autologous chondrocyte transplantation (ACT) and proposed application of medical remedies based on surface-active phospholipids. To this end we exposed chondrocyte to culture environments with mixtures of culture medium and phospholipid solutions.

Following various culture periods, cell survival and well-being were determined by measuring proliferation and assessing morphological features, and comparing these with the behaviour of cells grown in classical which were not mixed with surfactant, i.e., control culture medium.

Scanning electron microscopy and light microscopy demonstrate that the cells exposed to mixtures with surfactant were as healthy as those in the control environment with polygonal morphology, while proliferation assay indicated a noticeably higher level of proliferation over similar periods, for cells cultured in media that was mixed with surfactants. Also, the cells in media with unsaturated surfactants responded better than those cultured in mixtures containing saturated surfactant.

1. Introduction

Osteoarthritis (OA), the most common form of arthritis is a degenerative joint disease, which causes severe long-term pain and physical disability. The disease could initiate from the initially degeneration of the articular cartilage and then spread to the entire joint including subchondral bone (Brandt et al., 1998). OA is extremely prevalent in all populations with almost 1.3 million people suffering only in Australia (AIHW, 2007) and over 8 million people in the UK living with osteoarthritis (Yelin, 1992).

A community-based survey has shown that the incidence of OA increases by 2 to 10-fold from the ages of 30–65, and increases further after the age of 65 (Oliveria et al., 1995) indicating that OA is age related. Treatment options aim to return the affected joint to an acceptable level of mobility. The mainstay of current treatment is providing pain relief in an effort to delay the eventual need for total joint replacement (TJR). However, the demand for TJRs by younger patients has increased, and current prostheses have limited life span of between 10 and 25 years leading to the need for revision surgery.

Autologous chondrocyte transplantation (ACT) is one of the newer procedures in the treatment of cartilage defects in the younger patients. This is a cell-based tissue engineering method which attempts to regenerate or repair cartilage, using articular chondrocyte that have been isolated and grown *in vitro*. Using a patient's own chondrocytes is attractive because it raises the possibility of cartilage regeneration rather than just repair of a lesion. It has been observed that other than osteochondral transplantation (OCT), ACT is the only other method of treatment that currently provides the capacity for articular cartilage repair that can produce hyaline or hyaline-like tissue (Hangody et al., 1998).

It has also been observed that articular surfaces from hips and knees removed at joint replacement in patients with osteoarthritis were appreciably deficient in surface-active phospholipid (SAPL) (Hills et al., 1998). Chen et al. (2005) suggested that the application of phosphatidylcholines-based remedies could facilitate the treatment of osteoarthritis. Previous clinical study

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showed that a saturated phosphatidylcholine (SPC), dipalmitoylphosphatidylcholine (DPPC) was effective providing relieve for OA patients for 14 weeks (Vecchio et al., 1999).

There are two types of SAPLs in synovial fluid: (1) phosphatidylcholines (PCs) which predominate, and (2) non-phosphatidylcholines (e.g. phosphatidyl glycerol) which are present in smaller quantities. The phosphatidylcholines can be separated into saturated PCs (SPCs) and unsaturated species (USPCs) (determined by the presence of double bonds in their fatty acid chains). The unsaturated species are dominant in joints constituting approximately 92% of the total lipid content in these areas of the human skeleton (Hills and Butler, 1984; Schwarz and Hills, 1996).

Gale et al. (2007), Hills and Crawford (2003) and Jones et al. (2004) have studied the lubrication properties of unsaturated and saturated SAPL-based surfactants while there has also been a notable amount of work done on their influence on the mechanical characteristics of cartilage (Oloyede et al., 2004; Gudimetla et al., 2007). However the effect of SAPL on cells, which is crucial for any medical application involving the input of this substance into the body, has not been studied. More importantly, and because of the inevitability of contact between seeded chondrocytes in ACT and phospholipids in solution in the joint during treatment, we argue that it is now imperative investigate whether or not such contact is beneficial or deleterious to growing cells.

Therefore our study was designed to investigate and obtain insight into the response of cells to synthetic surface-active phospholipids and thereby obtain crucial information on the viability of ACT on a systemic basis, where the system investigated comprises of cells and phospholipids surfactant in close contact.

The system is evaluated by performing cell culture experiments where the standard culture medium is mixed with components of saturated and unsaturated SAPL in different proportions. Following the culture experiment, the proliferation and morphology of cells will be measured and compared to behaviour of cells in control culture environment.

2. Materials and methods

2.1. Materials

The culture medium and penicillin/streptomycin were purchased from Invitrogen Corporation Melbourne, Australia and the foetal calf serum was purchased from HyClone, Logan UT.

The following SAPL constituents were obtained from Sigma–Aldrich, Australia: palmitoyl-linoleoylphosphatidylcholine (PLPC), palmitoyl-oleoyl-phosphatidylcholine (POPC), stearoyl-linoleoylphosphatidylcholine (SLPC), dilinoleoyl-glycero-phosphocholine (DLPC) and the saturated dipalmitoyl-glycero-phosphocholine.

2.2. Isolation and culture of cells

Tissue samples were harvested from OA patients undergoing elective knee surgery, more precisely from femoral condyles and tibial plateaux, and cells were isolated. For this study, we selected three male patients aged 55 years (Patient A), 59 years (Patient B) and 69 years (Patient C). Although the tissue samples came from patients undergoing joint replacement, the chondrocytes were isolated from the visually normal section of the excised tissue. After harvesting, cartilage was enzymatically digested (collagenase II), chondrocytes were isolated and filtered to remove any undigested cartilage particles, then washed with buffer. Cells were then resuspended in Dulbecco's Modified Culture Medium (DMEM, Invitrogen Corporation, Melbourne, Australia); supplemented with 10% foetal calf serum (FCS, HyClone, Logan UT) and 1% penicillin/streptomycin (GIBCO, Invitrogen Corporation, Melbourne, Australia) in a standard humidified incubator at 37 °C containing CO_2/95%. Culture medium was changed every 2–3 days.

Upon reaching confluence, the cells were treated with trypsin and subcultured into 24-well plates for light microscopy and scanning electron microscopy and 96-well plates for the proliferation assay. Fifth-passage cells were used in all experiments.

2.3. Sample groups

Three different groups were investigated in this study, namely:

- A control group where chondrocytes were cultured with DMEM only for the entire period of the experiment, with medium changed every 3 days.
- A second group which consists of cells cultured in medium and SAPL mixtures from the outset, with medium and SAPL changed every 3 days.
- A third group which consists of cells cultured in medium for 3 days, before replacing it and adding surfactant.

The surfactant solutions were applied such that the solution to which cells were cultured were made of surfactant: culture medium ratios of 1:9, 1:4 and 1:2 and concentration of surfactant of 0.001, 0.002 and 0.01 g/l.

2.4. Analysis of cell response

Light microscopy, still photography, scanning electron microscopy and proliferation assays were employed to determine the proliferative responses and morphology of the cultured cells in all three experimental groups mentioned above. The results from these procedures practically indicated whether or not the surfactant, in the proportions mixed in the culture medium, had a noticeable harmful effect on the cells.

2.4.1. Light microscopy

Chondrogenic cells were seeded in 6-well and 24-well plates and examined daily on an inverted microscope. Selected islets of cells could be easily relocated on successive days and rephotographed at the lower power magnifications to show the changing appearance of the cell colonies.

2.4.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed to visualize the cell morphology and proliferation.

Chondrogenic cells were cultured for 15 day on glass coverslips held in 24-well culture plates with culture medium or mixture of culture medium and surfactant, which were changed every third day. Prior to scanning electron microscopy, the cells were fixed with 2.5% glutaraldehyde, washed several times with PBS, osmium tetroxides and distilled water, and were dehydrated in ascending alcohol concentration (50%, 70%, 90% and two times at 100% ethanol). The coverslips were then glued to the specimen stubs, critical-point dried, gold coated, and examined by SEM (FEI Quanta 200 Environmental, FEI Company).

2.4.3. Cell proliferation

Cell proliferation was measured using the WST-1 based colorimetric assay (Roche), which relies upon the ability of living cells to reduce a tetrazolium salt into a soluble coloured formazan product.

Primary chondrocytes were plated into microtiter plates (tissue culture grade, 96 wells, flat bottom) at an initial concentration of 5×10^3 cells/cm^2 in 100 μl culture medium [DMEM (Gibco, Invitrogen), supplemented with 10% foetal bovine serum (FBS, Qualified) and 1% penicillin/streptomycin] containing various amounts (formulas) of surfactant (as described above), while control cultures were not exposed to surfactant. All analyses were performed in triplicate.

Proliferation for up to 8 days of culture was evaluated by WST-1 assay (Roche Applied Science, Penzer, Germany). Prior reading, 10 μl/well of cell proliferation reagent WST-1 was added and the cells were incubated for 4 h at 37 °C, 5% CO_2. The WST-1 derived precipitate, produced by the metabolically active cells in the culture, was quantified by a microplate reader (Benchmark Plus Microplate Spectrophotometer, Bio-Rad Laboratories) at A_{440} as per manufacturer's protocol and the viable cells for each condition were determined by WST-1 absorbance. To examine cell proliferation, the average absorbance of wells containing the culture medium and WST-1 (considered as the background) was subtracted from the absorbance of the wells containing the cells, surfactant and/or culture medium and the WST-1.

2.4.4. Still photography

To determine whether or not the synthesized surfactant formula has a detrimental effect on cells, chondrogenic cells were cultured using standard culture medium (DMEM supplemented with 10% FBS and 1% penicillin/streptomycin) and compared to cells cultured in standard culture medium supplemented with surfactant using time and dosage as variables.

Still photography, via inverted microscope was used to arrive at a qualitative assessment of the effect of surfactant on the cultured cells and hence proliferation rate and morphological features.

3. Results

The light microscopy photographs (Fig. 1a and b) and scanning electron microscopy images (Fig. 2a and b) showed that the control