Phenotype and Genotype Analysis of Primary Articular Chondrocytes Sub-Cultured on Collagen Type 2 Crude Extract

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AIM OF THE STUDY

To test the phenotypic and genotypic effects of growing primary articular chondrocytes on Collagen Type II to determine whether the cartilage phenotype is maintained and dedifferentiation and fibrosis suppressed when compared to chondrocytes grown on Collagen Type I.

BACKGROUND

The organization of chondrocytes within cartilage differs depending on cartilage type and location within tissue. We are interested in articular chondrocytes specifically, for they are the source of cells for the cell-based treatment of cartilage lesions. The expansion of primary articular chondrocytes in monolayer culture is necessary to obtain sufficiently high numbers of cells for cell-based therapies such as Autologous Chondrocyte Implantation (ACI). A major problem associated with this approach is that chondrocytes undergo dedifferentiation into fibroblast-like cells during expansion. This process results in low quality tissue for transplantation. The current techniques used for chondrocyte expansion include growing them on Collagen Type I. However, in articular cartilage, the extracellular matrix substrate mainly present is Collagen Type II. We therefore hypothesized that growing articular chondrocytes in Collagen Type II might have beneficial effects on chondrocyte expansion, in addition to being more economical. In this project, a protocol involving extraction of Collagen Type II (Col2) from articular cartilage was devised and the effects of growing chondrocytes on it as opposed to Collagen Type I (Col1) were investigated.

METHODS

Isolation of Bovine Articular Chondrocytes

Primary bovine chondrocytes were isolated from fresh cartilage and 10^6 cells were cultured in chondrocyte culture medium on silicone rubber in modified six-well plates and microscope slides. Control experiments were conducted with Col1, the others with Col2 crude extract. The cells were grown for 6 days, and several studies were conducted on them, described in detail on Panels 1 – 3.

Collagen Type II Crude extraction

Fresh cartilage tissue was obtained from bovine knee joints and placed in a 2% SDS treatment @ 37ºC overnight, to decellularize the tissue (Figure 1). The tissue was then washed in PBS for 2 hrs @ 37ºC, and then broken down mechanically via a homogenizer. Subsequently, the cartilage was partially digested using Collagenase 2 (0.15 mg/ml, solution per 100 mg tissue). The crude extract is then ready to use.

Panel 1: Morphology, cell count, and viability assessment of chondrocytes grown for 6 days on Col1 and Col2 coated plates

Methods

- Morphology: chondrocyte morphology was assessed daily by light microscopy (Figure 2).
- Cell count: the number of cells present in each well of the 6-well plates was recorded (Figure 3).
- Viability: A live/dead assay was performed via immunostaining and assessed by fluorescent light microscopy (Figure 4).

Results

Figure 2. Representative picture of primary chondrocytes cultured in monolayer on Col1 and Col2 coated plates. Chondrocyte morphology shown for days 1 and 6 of culture.

Figure 3. Amount of cells present in each well of a 6-well plate coated with Col1 and Col2 at day 6 of culture.

Figure 4. Percent of total viability shown for chondrocytes grown on Col1 and Col2 coated plates, respectively.

Panel 2: Replicating vs. Apoptotic cells

Methods

On day 6 of cell culture, a study of proliferating vs. apoptotic cells was conducted using immunostaining with PH3 marker for cell proliferation and CASP3. Fluorescent light microscopy was used to evaluate the results (Figure 5).

Results

Figure 5. a) Comparison of cell number undergoing replication (PH3 marker) and cell death (CASP3 marker) as a percentage of total cells in plates coated with Col1 and Col2 (day 6).

b) Representative picture of immunostaining for replicating and apoptotic cells.

Panel 3: Gene expression indicative of chondrocyte-like behaviour and dedifferentiation

Methods

After 6 days of culture, the gene expression level of 5 genes was determined by quantitative PCR (Figure 6):

- Col2A1, Aggrecan, Sox9, and COMP are genes expressed by chondrocytes
- Col1A2 is a fibroblast-specific gene.

Results

Figure 6. Relative expression of bovine Col2A1, Sox9, Aggrecan, COMP and Col1A2 genes for chondrocytes cultured on Col2 coated plates compared to chondrocytes cultured on Col1 coated plates at day 6 of growth.

CONCLUSIONS

• Figures 2 – 6 indicate that thus far there is no substantial difference, morphological, in cell number, viability, or gene expression, observed in chondrocytes grown on Col2 coated plates as opposed to the controls grown on Col1 coated plates.

• Albeit there is no indication of a reduction in dedifferentiation, it was determined that Col2 extract can be employed instead of Col1.

• The advantage of employing Col2 extract is that it can simply be obtained in large quantities, can be reused, and is overall more economical than employing Col1.

• Additionally, a Col2 extract provides an environment for the chondrocytes that more closely resembles that of the live joint.

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