INFLUENCES OF DYNAMIC STRAIN AND SERIAL PASSAGE ON HUMAN DISTAL FEMUR CHONDROCYTES ISOLATED FROM TOPOGRAPHICALLY DISTINCT JOINT REGIONS APPLICABLE TO AUTOLOGOUS CELL TRANSPLANTATION

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Introduction

Autologous chondrocyte cell transplantation (ACCT) is currently used to repair defects in distal femur articular surfaces. In this repair strategy, chondrocytes are isolated from relatively low loaded areas (LLA) of the joint, culture expanded in monolayer and implanted predominantly in high loaded areas (HLA) of the joint. It is known that topographical variation in cell metabolism has been correlated to areas of joint loading and differential mechanical responses of superficial and deep cells have been reported. Little is known about the intrinsic behaviour of chondrocytes isolated from clinically relevant joint sites in particular, their response to amplification and dynamic mechanical stimulation at physiological strains and frequencies.

In this study, the authors test the hypotheses that a) cell metabolism will be significantly different dependent on origin b) basal metabolic variations will be maintained through monolayer amplification c) differential mechanical responses between LLA and HLA chondrocytes will be evident from freshly isolated cells (P0) and through successive passages (P1 and P2).

Methods

Pristine human distal femurs were obtained from 5 patients (ages 13-48 yrs) following amputation. Two distal femurs were used for the full study, three femurs were used for early study objectives. Cartilage tissue was harvested from LLA clinically used as biopsy sites in ACCT and from condylar regions typically prone to acute defects. Chondrocytes were isolated from full depth tissue by sequential pronase and collagenase digestion. A proportion of cells from LLA and HLA were seeded in monolayer at 4500 cells.mL\(^{-1}\) and cultured in DMEM+10% FCS through two confluent serial passages. Cells were released from monolayer with 0.2 % trypsin and further digested with 1 % pronase in DMEM for 1 hour to produce a single cell suspension.

Cells from P0, P1 and P2 were seeded into 4 % ultra low Tm agarose at 10 x 10^6 cells.mL\(^{-1}\). Six constructs (5 mm Dia x 5 mm deep) from both LLA and HLA chondrocyte populations were dynamically stimulated at 15 % strain, 1 Hz for 24 hours in 1 mL \(-1\) DMEM containing 10% FCS, 3.7 kBq (1µCi.mL\(^{-1}\)) 35SO\(_4\) and 3.7 kBq (1µCi.mL\(^{-1}\)) 3H-TdR. Control constructs were unstrained. Chondrocyte constructs and medium were analysed for 35SO\(_4\) and 3H-TdR incorporation, and for the synthesis of nitric oxide (NO) quantified in the form of stable nitrite, all normalised against DNA.

Results

The basal synthesis of NO in control of HLA and LLA cultures increased from P0 to P2 accompanying a reduced inhibitory effect of dynamic compression on NO synthesis (p<0.05). LLA cells at P0 were inhibited more by dynamic compression than HLA cells, however, with passage, HLA chondrocytes were inhibited more than LLA cells (figure 1, p<0.05). A large increase in the incorporation of both 3H-TdR and 35SO\(_4\) was evident at P2 in comparison with levels of P0 and P1 (p<0.05). Control levels of 3H-TdR and 35SO\(_4\) incorporation were significantly higher in the HLA chondrocytes than LLA chondrocytes for P0, P1 and P2 (figure 2). Mechanical inhibition of 3H-TdR and 35SO\(_4\) incorporation was evident at P0 for HLA and LLA cells (p<0.05). Mechanical induced up-regulation of 3H-TdR and 35SO\(_4\) resulted with P1 and P2, the greatest stimulation occurring for HLA chondrocytes at P0, P1 and P2 in terms of 35SO\(_4\) (p<0.05) and P2 for 3H-TdR incorporation (p<0.05).

Discussion

Differences in cell metabolism between HLA and LLA chondrocytes of P0 cultures were maintained through monolayer amplification. Both HLA and LLA chondrocytes had differential responses to dynamic mechanical compression in P0, P1 and P2 cultures. The results conclude that HLA chondrocytes, when mechanically stimulated, incorporate more 35SO\(_4\) and 3H-TdR while maintaining high levels of NO inhibition. NO has been inversely correlated to levels of 3H-TdR and 35SO\(_4\) incorporation and is strongly influenced by mechanical compression via the chondrocytes mechanotransduction mechanism(s).

The results strengthen the hypothesis that topographical differences in cell metabolism within the distal femur may be pre-determined and therefore implicating distinct cell populations within the distal femur topography. Mechanical loading of the joint may also contribute to the observed variations. In the context of ACCT, the full potential of repair may not be achieved since a population of cells that perceives relatively low stress in LLA’s may not perform adequately in high stress defect sites of the medial and lateral condyles. This may have implications for the efficacy of repair where proliferation, matrix synthesis, and mechanical responsiveness of the cells are of critical importance.

Figure 1 Effects of dynamic compression on the synthesis of NO, from two distal femurs (ages 13 & 24 yrs) values plotted as % over control (p<0.05)

Figure 2 Incorporation of 35SO\(_4\) in control cultures of HLA and LLA cells (13 & 24 yrs old), significance level (p<0.05)


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Poster Session - New Investigator Recognition Awards - Hall E

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