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Articular chondrocyte passage number: Influence on adhesion, migration, cytoskeletal organisation and phenotype in response to nano- and micro-metric topography

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Abstract

The isolation and culture of articular chondrocytes is a prerequisite of their use in tissue engineering, but prolonged culture and passaging is associated with de-differentiation. In this paper we studied the influence of nanometric and micrometric grooves (85 nm to 8 μm in depth and 2 μm to 20 μm in width) on 1st and 2nd passage ovine chondrocytes since our earlier findings indicate that primary cells are not affected by such features. 1st and 2nd passage chondrocytes cultured on grooved substrata showed a polarisation of cell shape parallel to the groove long axis and F-actin condensations were evident at groove ridge boundaries. An increase in cell migration with increasing groove depth was observed. Both passages of chondrocytes maintained type II collagen expression, but to a lesser degree in 2nd. This study demonstrates that passage number alters the response of chondrocytes to micrometric and nanometric topography, and could be important in ex vivo cartilage engineering.

Keywords: Topography; Ovine articular chondrocytes; Passage number; Cell adhesion; Cell motility; F-actin; Cell engineering

1. Introduction

Articular cartilage is a tissue with extraordinary biochemical and biomechanical properties, designed to provide the skeleton with free mobility whilst protecting the underlying bone from shear forces and friction (Silver and Glasgold, 1995; Von der Mark, 1986). As a result, an individual only becomes aware of its protective function when the cartilage is damaged, either traumatically, or as a consequence of joint degeneration (Chen et al., 1997). Cartilage has been shown to have a very limited capacity to repair itself, and the tissue formed in lesions is commonly a type I collagen rich fibrocartilage, which breaks down over time due to poor mechanical properties (Huckle et al., 2003). Current treatment modalities result in sub-optimal long term cartilage regeneration (Temenoff and Mikos, 2000). As a result attention has turned to cell and tissue engineering with the hope that it would be possible to develop alternative, long term treatments for cartilage defects (Vacanti et al., 1993).

Attempts to tissue engineer articular cartilage have primarily relied on the combination of terminally differentiated autologous chondrocytes with either natural or synthetic three-dimensional polymer scaffolds (Deng et al., 2002). The materials used to create three-dimensional scaffolds include polyglycolic acid, poly-lactic acid, poly (DL-lactic-co-glycolic acid), and collagen gels (Temenoff and Mikos, 2000). Many of these materials were selected either because their physical properties supported articular chondrocyte adhesion,

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three-dimensional tissue reconstruction, or because their chemical composition specifically maintained the chondrocyte phenotype (Seal et al., 2001). However, since the amount of cells which can be isolated from a biopsy is usually not sufficient to seed a construct at the required density, chondrocytes have to be passaged in monolayer culture. The dedifferentiation or loss of phenotype of articular chondrocytes with passage number is well documented (Kuroda, 1964; Kuettner et al., 1982), but has been perceived as a necessary tradeoff, and it is only recently that attention has turned as to how the passage number of chondrocytes affects their ability to re-constitute cartilage in three-dimensional constructs in bioreactors (Marijnissen et al., 2000).

The adhesion of a cell to a surface is influenced by factors such as surface chemistry and topography, both of which can further influence cell adhesion, cell shape, cytoskeletal organisation, signal transduction, migration and differentiation (Clark et al., 1987, 1990). The control of cell adhesion to surfaces impacts on the selection of the materials used in tissue engineering, especially surfaces which enhance or reduce adhesion are desirable (Chen et al., 1997). Many of the reports on cell reaction to topography were studies where either established cell lines or primary cells have been employed (Curtis and Wilkinson, 1998). We have previously shown that *primary* articular chondrocytes respond to 750 nm deep grooves, with increased levels of migration (Hamilton et al., 2005). In addition, we demonstrated that primary chondrocytes, react to multiple grooved substrata with neither alignment of the cell long axis, nor F-actin rearrangement, both of which are responses observed in nearly every other cell type tested so far on such substrata (Curtis and Wilkinson, 1998). There has, however, never been any report on how passage of cells in monolayer directly affects cellular response to topographies such as grooves, although we have previously shown that passaged chondrocytes are responsive to concave surfaces (Riehle et al., 1998).

Starting from our previous report on the non-typical response of primary ovine chondrocytes to multiple grooved substrata, we aimed to investigate whether the dramatic change in articular chondrocyte phenotype which occurs with passaging and goes hand in hand with the establishment of monolayer culture, is reflected in a change in chondrocyte behaviour on topographically designed substrata with multiple grooves.

2. Materials and methods

2.1. Fabrication of topography

Fused silica slides (1 mm thick) are cut into 25mm^2 samples. The silica is cleaned by soaking in a solution of 3:1 sulphuric acid:hydrogen peroxide for 5–10 min at 80 °C followed by a rinse in RO water, and then blown dry with filtered air. The silica is coated with S1818 photoresist (Shipley, Coventry, UK) by spinning at 4000 rpm for 30 s, followed by a soft bake at 90 °C for 30 min. This produces a resist thickness of 1.8 μm. The resist is then patterned by exposure to UV light for 10 s through a chrome mask patterned with the required grating pattern, using a mask aligner (HTG San Jose, CA). The exposed resist is removed by immersing the sample in a solution of 1:1 Shipley developer: RO water for 65–75 s, followed by rinsing in RO water and blown dry. The samples are dry-etched in a reactive ion etching unit (RIE80, Plasma Technology, Bristol, UK) at a rate of approximately 30 nm/min. One hundred watts of rf power at a frequency of 13.6 μHz powered a 15-cm diameter table; CHF_3 was used at a flow rate of 20 sccm and a pressure of 15 mT. The self-bias was removed in warm acetone and all samples were etched as described above for an additional minute without protection to ensure uniform surface chemistry.

2.2. Cell isolation

Isolation of articular chondrocytes from ovine metacarpal-phalangeal joints was performed aseptically. Cartilage was dissected from the joints of 8-month-old lambs obtained from a local slaughterhouse and stored overnight at 37 °C in culture medium (DMEM supplemented with 10% foetal calf serum, 3 mM glutamine,100 U/ml penicillin, 100 μg/ml streptomycin, 2.5 μm/ml amphotericin B) plus 67 U/ml of nystatin (DMEM, Sigma Chemicals, Poole, Dorset). The tissue was chopped into 1–4 mm fragments and washed three times with culture media. The medium was removed and the cartilage was gently shaken in a solution of 1% pronase (Sigma Chemical) in culture medium, followed by type 1A collagenase at a concentration of 300 units/ml (Type 1A, Sigma Chemicals) in culture medium for 3 h at 37 °C. The resulting cell suspension was passed through a 70 μm nylon cell strainer (Falcon/Becton Dickinson, Cowley, Oxford, UK). The filtrate was then transferred into centrifuge tubes and spun at $173 \times g$ for 5 min. The resulting pellet was washed three times and then resuspended in culture medium. The cells were plated in 75 cm^2 culture flasks at a density of 50×10^3 cells/ml, and fresh medium was added to the cells every 3 days.

2.3. Analysis of cell orientation and alignment

To calculate cell orientation and alignment on grooved substrates, cells were fixed in 4% formalin (Sigma, Poole, UK) in PBS for 5 min and then stained with Coomassie Blue for 5 min. Ten fields of view were captured from experimental and control surfaces using