Compartmentalization of the matrix formed by nucleus pulposus and annulus fibrosus cells in alginate gel

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Abstract

Intervertebral disc cells cultured in alginate gel are capable of reforming in alginate, a matrix that consists of two compartments: a rim of metabolically active cell-associated matrix and a more abundant, but metabolically less active, further removed matrix. At any one age and in most species, the cell-associated matrix formed by a nucleus pulposus or annulus fibrosus cell cultured in this way is less abundant than that formed by an articular chondrocyte. In both the cell-associated matrix and further removed matrix, the ratio of aggrecan to collagen is significantly higher in the case of nucleus pulposus than of annulus fibrosus, a feature that also distinguishes the matrices of the nucleus pulposus and annulus fibrosus in vivo. Nucleus pulposus and annulus fibrosus cells from older donors show a decreased ability to reform a cell-associated matrix rich in aggrecan. There is, however, some evidence that gene therapy and/or exposure of the cells to defined stimulatory factors can help overcome some of these age-related limitations. This contention is supported by recent evidence that nucleus pulposus and annulus fibrosus cells from adult donors can be manipulated to form, using the recently developed alginate-recovered chondrocyte system, a resilient tissue that bears many of the characteristics of the tissue in which these cells reside in vivo.

Culture of chondrocytes and intervertebral disc cells in alginate gel

In 1992, Maldonado and Oegema [1] reported that canine intervertebral disc cells cultured in alginate...
gel maintain their three-dimensional morphology and phenotypic stability for as long as 2 weeks. At approximately the same time, an alginate bead culture system was being refined in an attempt to identify differences in the organization and turnover of aggrecan in two matrix compartments: the cell-associated matrix, a highly structured compartment encircling each cell, and the further removed matrix, a loosely organized voluminous compartment lying between the cell-associated matrix areas [2,3].

In recent years, several studies have taken advantage of the newly refined alginate bead system to determine if the chondrocyte-like cells from the nucleus pulposus and annulus fibrosus similarly formed and turned over a compartmentalized matrix in the alginate beads [4]. In this brief overview, we highlight some of the key findings of those studies. We also draw attention to recent preliminary evidence that adult nucleus pulposus and annulus fibrosus cells precultured in alginate gel and then further maintained on a semi-permeable membrane are capable of reforming in vitro a resilient tissue that resembles the tissue from which the cells were isolated [5].

Advantages of the alginate bead culture system
The suspension of adult articular chondrocytes in alginate beads offers some clear advantages over other culture systems. The alginate gel is highly negatively charged and therefore mimics the extracellular environment of the proteoglycan-rich matrix [6]. It provides an environment in which the cells can orient themselves three-dimensionally and maintain phenotypic stability during months of culture [7]. Another advantage of the system is that the gel can be rapidly solubilized by the addition of calcium-chelating agents, making it possible to readily recover viable cells. Mild centrifugation after this solubilization step yields two fractions that contain macromolecules derived from two distinct compartments: the cell-associated matrix in the pellet and the further removed matrix in the supernatant [3,6]. It is worth noting that it is possible to fully remove traces of the alginate, something that is not as easy to achieve in the case of agarose [8].

The ability to rapidly and fully separate molecules in the two compartments of the matrix formed by adult articular chondrocytes in alginate gel made it possible to ask questions one was not able to address with other culture systems. For example, researchers attempting to determine whether aggrecan molecules residing in the cell-associated matrix are turned over at a different rate from their counterpart in the further removed matrix, were able to show that turnover is several fold more rapid in the cell-associated matrix compartment [3,6,8,9].

More recently, several studies have not only confirmed the original finding of Maldonado and Oegema [1] that alginate gel is also a useful medium to study the metabolism of intervertebral disc cells, but they extended them [4,10-14]. Some highlights of that research on intervertebral disc cells are presented below.

Formation and maintenance of a cell-associated matrix by intervertebral disc cells cultured in alginate
As reported for articular chondrocytes [15], adult intervertebral disc cells undergo a limited degree of cell division in alginate, but this is mostly limited to the first week in culture, prior to the development of pyridinium cross-links within the cell-associated matrix [4]. How often the cells divide varies with the age of the donor and from species to species. When compared with articular chondrocytes from the same donor, intervertebral disc cells are more sluggish in reforming a cell-associated matrix in alginate gel. The morphological appearance of nucleus pulposus and annulus fibrosus cells and the types of proteoglycan and collagen molecules they synthesize in alginate gel support the view that their phenotypes are more closely related to that of chondrocytes than that of meniscal cells, which synthesize relatively larger amounts of collagen type I and small non-aggregating proteoglycans [16,17].

Adult nucleus pulposus cells are less effective than annulus fibrosus cells in surrounding themselves with this ring of matrix [4]. When compared with annulus fibrosus cells cultured for the same amount of time under the same conditions, the rim of the cell-associated matrix surrounding nucleus pulposus cell clusters usually is significantly thinner, and its contents of proteoglycan, mostly aggrecan, and collagens, mostly type II, are lower [4]. These differences were shown to reflect the ability of the annulus fibrosus cells not only to synthesize more aggrecan molecules but also to incorporate them more effectively in the reforming
cell-associated matrix. Interestingly, a small proportion of intervertebral disc cells, but especially cells from the nucleus pulposus, fails to show evidence of a histologically detectable cell-associated matrix, even after several weeks in culture [4]. This supports the contention that the intervertebral disc contains several phenotypically different cell populations [18,19].

The cell-associated matrix probably plays a key role in situ by cushioning the forces applied during loading, thereby minimizing cell deformation and its potentially injurious effects [15]. In the cell-associated matrix formed in alginate by adult bovine and human articular chondrocytes, aggrecan is tightly packed within heterotypic cross-linked collagen fibrils [20], reaching a steady-state concentration that actually is higher than the mean concentration of aggrecan in the matrix in situ [15]. The cross-linked fibrillar network and entrapped resilient aggrecan molecules thus offer the cell a unique double protection from deformation during load bearing [4]. Studies are in progress to determine why intervertebral disc cells are less effective in reforming this type of protective shell. The relatively weak ability of intervertebral disc cells, especially nucleus pulposus cells, to reform, de novo, a cell-associated matrix rich in proteoglycans and cross-linked collagens is worth noting as it may help explain why degeneration of these tissues sometimes occurs early in life and why the degenerative changes are often progressive [4] (Figure 1).

**Differences in the rate of turnover of the cell-associated and interterritorial (further removed) matrix compartments: changes during aging**

One of the major advantages of the alginate bead system is that it allows precise measurement of the half-life of matrix molecules in the cell-associated matrix and further removed matrix. As a result, it is now clear that aggrecan molecules that become established in the cell-associated matrix, close to the membrane of adult bovine and human articular chondrocytes, turn over much more rapidly than those that reach the further removed matrix [6,15].

More recently, Masuda et al. [21] made a similar observation in a comparative study of rabbit adolescent nucleus pulposus and annulus fibrosus cells cultured in alginate. They noted that the half-life of $^{35}$S-aggrecan molecules retained in the cell-associated matrix was shorter than the mean half-life of $^{35}$S-aggrecan molecules synthesized by the cells (Table 1). These investigators also showed that interleukin-1α, at a concentration of 1 ng/ml, was more effective in causing an acceleration in the rate of degradation of $^{35}$S-proteoglycan molecules in the cell-associated matrix than in the further removed matrix.

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**Figure 1**

**Schematic representation of the importance of the cell-associated matrix in protecting cells against forces applied to the tissues during loading**

Chiba et al. [4] postulated that the cell-associated matrix (CM) rich in cross-linked collagen fibrils and resilient proteoglycans acts as a shell that cushions the external forces and stresses and thereby protects individual cells from severe deformation. The presence of a more abundant cell-associated matrix around annulus fibrosus (AF) than nucleus pulposus (NP) cells would, according to this hypothesis, offer better protection against the deleterious effects of cell deformation. From Chiba, K., Anderson, G. B., Masuda, K. and Thonar, E. J. (1997) Metabolism of the extracellular matrix formed by intervertebral disc cells cultured in alginate. Spine 22(24), 2885–2893. Used with permission.
Table 1

Half-life of $^{35}$S-proteoglycans synthesized by nucleus pulposus and annulus fibrosus cells from adolescent rabbits in alginate beads

In both cases, the half-life of proteoglycans that had become resident in the cell-associated matrix was considerably shorter than the average half-life of all proteoglycans in the beads. The results also show that the addition of interleukin-1 (IL-1α) at 1 ng/ml to the medium had the greatest effect on proteoglycans residing in the cell-associated matrix. Data taken from [21].

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<tr>
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<th>Nucleus pulposus</th>
<th>Annulus fibrosus</th>
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<tbody>
<tr>
<td>Cell-associated matrix</td>
<td>Control</td>
<td>IL-1α</td>
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<tr>
<td></td>
<td>12.6</td>
<td>4.5</td>
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<td>Whole beads</td>
<td>27.7</td>
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The alginate-recovered-chondrocyte (ARC) approach to the study of intervertebral disc matrix formation and repair

In what constitutes a breakthrough for scientists interested in studying cartilage formation and repair, Masuda et al. [22–24] developed a novel two-step culture method for the production of cartilaginous tissue in vitro. The first step consists of culturing phenotypically stable chondrocytes under conditions optimal for the formation of the cell-associated matrix. The second step allows these cells, with their matrix, to rapidly form a solid cartilage mass on a tissue-culture insert with a porous membrane. Tissue engineered over a period of 2–3 weeks by this ARC method is softer than normal cartilage: it is rich in aggrecan but has an immature collagen network [25]. These properties should enable surgeons to press-fit the tissue into a cartilage defect and promote integration within the host tissue. In addition, separate time-course studies showed that the contents of collagen and collagen-specific cross-links increase with time in culture, suggesting that better integration can be expected after the transplantation to the defect. Most importantly, this ARC method also makes it possible to study important metabolic processes in matrix repair and to identify ways and means of promoting the repair processes to reform a tissue that has good mechanical properties.

Matsumoto et al. [5] recently showed that this approach could be used to induce the formation of a disc-shaped tissue by adult bovine intervertebral disc cells cultured in the presence of osteogenic protein-1 (Figure 2). The collagen content was higher and the ratio of proteoglycan/collagen was lower in the annulus fibrosus than in the nucleus pulposus tissue. This, and the results of the analysis of the compressive and tensile properties of the tissues, suggested that annulus fibrosus cells thus cultured form a more fibrous tissue than nucleus pulposus cells. These findings suggest that intervertebral disc tissues may be engineered in vitro using different cell sources. More importantly, they offer preliminary evidence that this does not require the use of either a natural or synthetic scaffold.

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References


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