Specific interaction between cartilage proteoglycans and hyaluronic acid at the chondrocyte cell surface

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Binding of exogenous [^{35}S]sulphate-labelled cartilage proteoglycans to cells was studied with suspension cultures of calf articular-cartilage chondrocytes. Proteoglycans interact with hyaluronic acid at the cell surface via their hyaluronic acid-binding region. The binding is time-dependent and saturable, but does not appear to be freely reversible. The bound ^{35}S -labelled proteoglycans are located at the cell surface, and only small proportions of the proteoglycans are internalized.

Chondrocytes cultured *in vitro* retain their capacity to synthesize and secrete proteoglycans. *In vivo* as well as *in vitro* most of the proteoglycans participate in the extracellular formation of large aggregates together with hyaluronic acid and link protein (Hascall & Heinegård, 1974*a,b*; Kimura *et al.*, 1979, 1980; Björnsson & Heinegård, 1981*b*). A substantial proportion of the proteoglycans is, however, associated with the cell surface, even in single-cell suspension cultures (Björnsson & Heinegård, 1981*a*). The relative size of this cell-associated pool varies with the culture system used, and the mechanism for the binding of proteoglycans to the chondrocyte cell surface is not known. The turnover of these proteoglycans is slow (Mikuni-Takagaki & Toole, 1979; Björnsson & Heinegård, 1981*a*), making it unlikely that the pool of proteoglycans at the cell surface only represents an intermediate step in the export into the medium.

Binding of proteoglycans to cells has been studied with other cell types. In the case of heparan sulphate proteoglycans bound to the surface of hepatocytes, the interaction is mediated by two different mechanisms. One fraction binds via the polysaccharide side chains to a cell-surface receptor, and another is bound via a hydrophobic region in the proteoglycan core protein, which is believed to be integral in the membrane (Kjellén *et al.*, 1980, 1981). Other studies have shown that fibroblast proteoglycans can bind to fibroblasts, probably via their protein core (Prinz *et al.*, 1978). These proteoglycans were internalized, however, and therefore represent a degradation pool rather than a structural one.

In the present investigation we have studied the ability of chondrocytes in suspension culture to bind exogenous cartilage proteoglycans. Such binding of proteoglycans to the chondrocyte surface could be

an important part of a feedback control on chondrocyte cell function exerted by the extracellular matrix.

Experimental

Materials

The medium used for spinner culture was Ham's F12 medium supplemented with 50× essential amino acids (for Eagle's minimum essential medium; Flow Laboratories, Rockville Pike, MD, U.S.A.) (20 ml/l), dextran sulphate (20 µg/ml) (Pharmacia Fine Chemicals, Uppsala, Sweden), penicillin (Flow Laboratories) (50 i.u./ml) and streptomycin (Flow Laboratories) (50 i.u./ml) and buffered with 20 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (Sigma Chemical Co., St. Louis, MO, U.S.A.), pH 7.4. For binding experiments Ham's F12 medium buffered with 20 mM-Hepes at pH 7.4 was used. Collagenase CLS was from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.), and *Streptomyces* hyaluronidase was from Miles Laboratories (Elkhart, IN, U.S.A.). Conical polypropylene tubes (50 ml) and bacteriological Petri dishes were from Nunc (Roskilde, Denmark). For binding experiments 2 ml polypropylene vials (no. 72.708; Sarstedt, Numbrecht, W. Germany) for the Eppendorf 3200 centrifuge were used.

Articular-cartilage proteoglycans were prepared from metacarpophalangeal joints of 6-month-old calves. These proteoglycans were extracted with 4 M-guanidinium chloride/5 mM-*N*-ethylmaleimide/50 mM-sodium acetate buffer, pH 5.8, containing the protein inhibitors 5 mM-benzamidinium chloride, 0.1 M-6-aminohexanoic acid and 10 mM-EDTA. Solid CsCl was added to the extract to increase the density to 1.50 g/ml. The proteoglycans were isolated by equilibrium centrifugation in an MSE

6 × 14 ml swinging-bucket rotor at 30000 rev./min (110000 g_{av}) for 65 h at 18°C. The bottom quarter of the tube was separated with a Beckman tube slicer. This fraction had a density of 1.61 g/ml and contained the bulk of the proteoglycans. Nasal-septum-cartilage proteoglycan aggregates, hyaluronic acid-binding region and a mixture of chondroitin sulphate-attachment region and keratan sulphate-attachment region were prepared as described previously (Heinegård & Axelsson, 1977). Human umbilical-cord hyaluronic acid was from Sigma Chemical Co. Hyaluronic acid oligomers were prepared by the method of Hascall & Heinegård (1974b).

Isolation of cells

Articular-cartilage chondrocytes were prepared from metacarpophalangeal joints from forelegs of 6-month-old calves. Hyaline cartilage was dissected from all articular-cartilage surfaces of the joint and cut into small pieces. The cell-suspension procedure was essentially the one described by Björnsson & Heinegård (1981a) for the preparation of foetal-calf tracheal chondrocytes. In short, the chondrocytes were liberated by collagenase digestion (0.2%, w/v) under constant agitation for 9 h at 37°C. The cells were washed and transferred to spinner bottles containing Ham's F12 medium with supplements. The cells were kept in spinner culture at 37°C for 2 days to allow them to reconstitute their cell surface.

Preparation of [^{35}S]sulphate-labelled proteoglycans

Chondrocytes (0.5×10^6 cells/ml) were cultured in Ham's F12 medium containing 20 mM-Hepes, pH 7.5, and 50 i.u. of penicillin/ml on hydrophobic bacteriological Petri dishes. Carrier-free [^{35}S]sulphate (The Radiochemical Centre, Amersham, Bucks., U.K.) (20 μ Ci/ml) was added. After 6 days the medium was collected and concentrated by ultrafiltration (PM10; Amicon, Lexington, MA, U.S.A.). An equal volume of 8 M-guanidinium chloride was added to the concentrated medium, followed by solid CsCl to give a density of 1.50 g/ml. The ^{35}S -labelled proteoglycans were recovered from the bottom quarter of the tubes after equilibrium centrifugation, as described above. Proteoglycan preparations were dialysed against water, freeze-dried and dissolved in medium to make a concentrated stock solution for use in binding experiments.

Binding experiments

Cells were collected from spinner culture by centrifugation, washed twice with Ham's F12 medium, and suspended in fresh medium (containing no dextran sulphate) at a concentration of 2×10^6 cells/ml essentially as described previously

(Björnsson & Heinegård, 1981b). Samples (1 ml) of the cell suspension were pipetted into 2 ml polypropylene vials that had been precoated with bovine serum albumin (1 mg/ml in distilled water for 15 h at 20°C) to minimize non-specific binding to the walls. The cells were incubated for 1.5–2 h on a gyrating board (Nutator; Becton and Dickinson Labware, Oxnard, CA, U.S.A.) before the start of the experiment.

Addition of labelled proteoglycans was done either by pipetting 10 μ l of a concentrated stock solution directly into the cell suspension or by suspending cells that had been pelleted (30 s in an Eppendorf model 3200 centrifuge operated at 90 V) in 1 ml of fresh medium containing labelled proteoglycans. The amounts of proteoglycan bound were similar in the different procedures. When desired, competitors for binding were also added to the medium containing labelled proteoglycans. After incubation at 37°C on the gyrating board, the cells were pelleted by centrifugation and the medium was removed for determination of radioactivity. The cells were washed once by resuspending them in fresh medium followed by centrifugation, and this rinse was discarded. The efficiency of the rinsing procedure would allow less than 0.05% of non-bound proteoglycans to be recovered with the cell pellet. This pellet was finally suspended in 1 ml of Dulbecco's phosphate-buffered saline, pH 7.3 (Flow Laboratories), and samples were taken for determination of radioactivity. Before liquid-scintillation counting, samples of media and cells were made 1% (w/v) with respect to sodium dodecyl sulphate, heated at 60°C for 30 min and mixed with scintillation 'cocktail' (Instagel; Packard, Downers Grove, IL, U.S.A.) in a ratio of 1:10.

Results and discussion

Characterization of [^{35}S]sulphate-labelled proteoglycans

Preparations of ^{35}S -labelled proteoglycans were characterized by gel chromatography on Sepharose 2B. The major proportion of the proteoglycans was eluted as high- M_r monomers (Heinegård, 1972) (Fig. 1a). The ability of these labelled monomers to form aggregates with hyaluronic acid was demonstrated by the shift of a large proportion to the void volume after incubation with hyaluronic acid (2%, w/w) (Fig. 1b). The presence of the keratan sulphate-attachment region, typical for cartilage proteoglycans (Heinegård & Axelsson, 1977), was demonstrated by gel chromatography on Sepharose 6B of labelled proteoglycans sequentially digested with chondroitinase ABC and trypsin (results not shown). The ^{35}S -labelled proteoglycan preparations used in this study thus contained typical cartilage proteoglycan monomers capable of forming aggregates

Fig. 1. *Sepharose 2B chromatography of [³⁵S]sulphate-labelled proteoglycans*

(a) The isolated ³⁵S-labelled proteoglycans and carrier bovine tracheal-cartilage proteoglycan monomer were dissolved in 0.5 M-sodium acetate buffer, pH 7.0. The sample was chromatographed on a column (140 cm × 0.8 cm) of Sepharose 2B eluted with 0.5 M-sodium acetate buffer, pH 7.0. (b) Hyaluronic acid was added [2% (w/w) of proteoglycan] to an identical sample and left at room temperature for 24 h before chromatography as above. V₀, Void volume; V_t, total volume.

with hyaluronic acid. With time in storage the properties of the ³⁵S-labelled proteoglycans changed, as demonstrated by impaired aggregation with hyaluronic acid as well as decreased cell binding. This could possibly be due to radiolysis.

Kinetics of binding of [³⁵S]sulphate-labelled proteoglycans to cells

In initial experiments it was shown that when the ³⁵S-labelled proteoglycans were incubated with chondrocytes in suspension culture about 5% of the added molecules were bound and could be recovered with the cells (Fig. 2). The amount of ³⁵S-labelled proteoglycans that was bound to the chondrocytes increased rapidly during the first 2 h of incubation. The rate at which additional proteoglycans bound then decreased, and maximum binding was slowly reached within 5 h.

Fig. 2. *Time course of binding of [³⁵S]sulphate-labelled proteoglycans*

Cells were pelleted by centrifugation and resuspended in 1 ml of medium containing ³⁵S-labelled proteoglycans (○, 1 µg/ml, ●, 5 µg/ml). The amount of ³⁵S-labelled proteoglycans associated with cells was determined after incubation as described in the Experimental section.

Localization of bound [³⁵S]sulphate-labelled proteoglycans

To determine to what extent the bound proteoglycans were cell-surface-associated, the cells were allowed to bind ³⁵S-labelled proteoglycans for 2–5 h and were then treated briefly with trypsin (for details see Fig. 3 legend). At all times more than 86% of bound ³⁵S label was released by the digestion (Fig. 3), indicating a cell-surface localization of bound ³⁵S-labelled proteoglycans. Only a minor proportion was resistant to trypsin digestion and was recovered with the cell fraction, possibly representing internalized proteoglycans. This supports earlier studies on endocytosis of proteoglycans by chicken chondrocytes (von Figura *et al.*, 1980). These cells bound cartilage proteoglycans, which, however, were not internalized.

Concentration-dependence of binding of [³⁵S]sulphate-labelled proteoglycans

To investigate if the capacity of the chondrocytes to bind proteoglycans is saturable, various amounts of ³⁵S-labelled proteoglycans were added to 2 × 10⁶ cells in 1 ml of medium and incubated for 2 h. There was no sharp transition in amount bound when increasing amounts of ³⁵S-labelled proteoglycans were added (Fig. 4a). If the relative proportion of added proteoglycans bound to cells is considered, rather than the absolute amount, there was a marked

Fig. 3. Localization of bound [^{35}S]sulphate-labelled proteoglycans

^{35}S -labelled proteoglycans were allowed to bind to chondrocytes for the times indicated. The cells were then washed with fresh medium as described in the Experimental section, and resuspended in Ham's F12 medium containing 0.1% trypsin. Digestions were performed for 15 min at 3°C on a Nutator to prevent aggregation of the cells. Cells (trypsin-resistant material, $\bigcirc$) and medium (trypsin-releasable material, $\bullet$) were separated by centrifugation. The cells were then suspended in Dulbecco's phosphate-buffered saline, and samples of cell suspension and medium were taken for determination of radioactivity. Viability was better than 95% after trypsin treatment, as judged by Trypan Blue exclusion.

decrease in the proportion bound at concentrations above $16\mu\text{g}/\text{ml}$ (Fig. 4b). It appears that the saturation of the binding is not absolute, but that, when a certain number of proteoglycans are bound, additional molecules bind with less affinity, and possibly by other mechanisms. If the binding of proteoglycans to the cell surface is part of the construction of an extracellular matrix, no sharp division between cell-surface proteoglycans and extracellular matrix should be expected. In agreement, electron microscopy of articular-cartilage cells in monolayer culture shows a diffuse layer containing proteoglycans and collagen at the cell surface of the chondrocytes, indicating establishment of an extracellular matrix (Kuettnner *et al.*, 1982).

Specificity of binding of [^{35}S]sulphate-labelled proteoglycans

Unlabelled proteoglycans, isolated proteoglycan substructures and hyaluronic acid were used as competitors for the binding of ^{35}S -labelled proteoglycans to chondrocytes (Fig. 5). Both articular-cartilage and nasal-septum-cartilage (results not shown) proteoglycan monomers efficiently inhibited binding of the ^{35}S -labelled proteoglycans (Fig. 5a). About $50\mu\text{g}$ of articular-cartilage monomer is needed to give 50% inhibition of binding when $4\mu\text{g}$ of ^{35}S -labelled proteoglycans is present. One possible explanation for this is that these two preparations contain different proportions of two or more types of proteoglycans that differ in their cell-binding properties. Nasal-septum-cartilage proteoglycan aggregate

Fig. 4. Saturation of binding of [^{35}S]sulphate-labelled proteoglycans to chondrocytes

^{35}S -labelled proteoglycans were added by resuspending pelleted cells in medium containing ^{35}S -labelled proteoglycan at the concentrations indicated. After 2 h the incubation was terminated as described in the Experimental section. (a) Absolute values for bound ^{35}S -labelled proteoglycan. (b) Proportion of ^{35}S -labelled proteoglycan bound expressed as percentage of the amount added.

preparation (A1) containing mainly link-protein-stabilized aggregates was a less efficient competitor (Fig. 5b). Compared with proteoglycan monomers, about 7 times more (by wt.) of the proteoglycan aggregate preparation was needed to achieve 50% inhibition of binding of ^{35}S -labelled proteoglycans. Nasal-septum-cartilage A1 preparations have been shown to contain about 15% proteoglycan monomers (Heinegård & Hascall, 1979), since a substantial portion of these monomers can interact with hyaluronic acid *in vitro*, it appears that the inhibition obtained with this preparation could be due solely to the presence of free monomer.

Addition of reduced and alkylated articular-cartilage proteoglycan monomer ($64\text{ }\mu\text{g/ml}$) decreased the binding of ^{35}S -labelled proteoglycans to 75% of the control value, whereas intact monomers at this concentration decreased binding to 40% (Fig. 5a). Because reduction of monomers will only modify the hyaluronic acid-binding region (Heinegård, 1977), it appeared that this structure may be involved in the binding to cells.

Various substructures of proteoglycan monomer were used in the competition assay to identify the part of the molecule that binds to the cell surface.

Isolated hyaluronic acid-binding region competed efficiently with the ^{35}S -labelled proteoglycans for binding (Fig. 5c). On the other hand, a preparation containing a mixture of chondroitin sulphate-attachment region and keratan sulphate-attachment region (A1:T:A1; Heinegård & Axelsson, 1977) did not compete for binding (Fig. 5d). A somewhat increased binding of ^{35}S -labelled proteoglycans in the presence of chondroitin sulphate-attachment region and keratan sulphate-attachment region seems to be a general effect of anionic polymers, since dextran sulphate gave a similar, but even more pronounced, effect (Fig. 5g).

The hyaluronic acid-binding region was the only efficient competitor among the proteoglycan substructures, suggesting that the ^{35}S -labelled proteoglycans bind to the cell by this part of the molecule. Furthermore, when ^{35}S -labelled proteoglycans were reduced and alkylated to unfold the hyaluronic acid-binding region, binding was decreased to 2% compared with that of untreated proteoglycans (results not shown). Assuming, then, that proteoglycans bind to chondrocytes by their hyaluronic acid-binding region, a probable receptor structure is hyaluronic acid at the cell surface. If so, hyaluronic

Fig. 5. Competition for binding of ^{35}S sulphate-labelled proteoglycans

Additions of $4\text{ }\mu\text{g}$ of ^{35}S -labelled proteoglycan and competitors were made simultaneously by change of medium as described in the Experimental section. Incubation was for 3 h. ■, Control without competitors added. Bars indicate $\pm$ s.d. ($n=3$). (a) ●, Articular-cartilage monomer preparation; ○, reduced and alkylated articular-cartilage monomer preparation; (b) nasal-septum-cartilage aggregate; (c) hyaluronic acid-binding region; (d) chondroitin sulphate-attachment region and keratan sulphate-attachment region; (e) hyaluronic acid; (f) hyaluronic acid oligomers containing indicated number of monosaccharide residues; (g) dextran sulphate.

acid should be an efficient competitor, as was indeed the case (Fig. 5e). Further support for this mechanism for attachment was obtained by determining the size of hyaluronic acid required for efficient competition. The decasaccharide was the smallest hyaluronic acid oligosaccharide to compete efficiently (Fig. 5f), exactly as has been previously described for binding of proteoglycan monomer to hyaluronic acid in the formation of cartilage proteoglycan aggregates (Hardingham & Muir, 1973; Hascall & Heinegård, 1974b).

*Release of cell-bound [^{35}S]sulphate-labelled proteoglycans by treatment with *Streptomyces hyaluronidase**

The role of hyaluronic acid at the cell surface in the binding of ^{35}S -labelled proteoglycans was further studied by specific enzymic degradation. Digestion of chondrocytes with *Streptomyces hyaluronidase* released more than 95% of ^{35}S -labelled proteoglycans that had been bound to the cells (Table 1). To inhibit the proteolytic activity known to be present in the hyaluronidase preparation (Caputo *et al.*, 1980), ovomucoid plus newborn-calf serum, known to contain several proteinase inhibitors, was included in digestion buffers. Under such conditions the ^{35}S -labelled proteoglycan was not degraded during digestion, as judged by Sepharose 2B chromatography (results not shown). Furthermore, the aggregating capacity of the ^{35}S -labelled proteoglycans remained. No proteolytic activity was

detected in the mixture of hyaluronidase and proteinase inhibitors, with [^3H]acetyl-labelled haemoglobin as the substrate. The detection limit of this assay is low, corresponding to 0.1 ng of trypsin/ml. A 100-fold higher amount of trypsin (10 ng) than the highest possible proteinase activity of the digestion mixture containing 1 turbidity-reducing unit of hyaluronidase did not release any ^{35}S label from the cells (Table 1). The almost complete removal of bound ^{35}S -labelled proteoglycans by digestion with *Streptomyces hyaluronidase* demonstrates that the ^{35}S -labelled proteoglycans are indeed bound to hyaluronic acid at the cell surface.

Reversibility of binding of [^{35}S]sulphate-labelled proteoglycans to chondrocytes

The binding of proteoglycans to hyaluronic acid in proteoglycan aggregates is stabilized by link protein (Heinegård & Hascall, 1974; Kimura *et al.*, 1979; Hardingham, 1979). Such link-protein-stabilized aggregates are stable to competition with hyaluronic acid or proteoglycan monomers. Proteoglycans at the cell surface of chondrocytes have a slow turnover (Mikuni-Takagaki & Toole, 1979; Björnsson & Heinegård, 1981b). It is therefore possible that proteoglycan binding to hyaluronic acid at the chondrocyte cell surface is stabilized, possibly by link proteins. If so, they should not be readily displaced by either exogenous or newly synthesized proteoglycans. To investigate this possibility, ^{35}S -labelled proteoglycans were allowed to bind to the cells for 1 h and 4 h. Attempts were then made to displace bound proteoglycans by the addition of fresh medium containing the same competitors as used before (Fig. 6). In control cultures, bound ^{35}S -labelled proteoglycans decreased to 75% of the initial value during 3 h, i.e. 25% of bound proteoglycans were released. Competitors added to the cells at a concentration that would effectively compete for the initial binding resulted in release of only 39% of the ^{35}S -labelled proteoglycans. There was no significant difference between the results when ^{35}S -labelled proteoglycans were allowed to bind for 1 h or 4 h, other than the total amount bound. Thus binding of ^{35}S -labelled proteoglycans is not a freely reversible process. It is likely that the binding is stabilized by an additional factor, possibly link proteins.

General discussion

The present study has shown that cartilage proteoglycans bind via their hyaluronic acid-binding region to hyaluronic acid at the chondrocyte cell surface and that the binding is stable towards competition. It is possible that this hyaluronic acid-proteoglycan complex is stabilized by link proteins, in analogy to the proteoglycan aggregates

Table 1. Release of cell-bound [^{35}S]sulphate-labelled proteoglycan by *Streptomyces hyaluronidase*

Labeled proteoglycan (4 μg) was allowed to bind to chondrocytes in 1 ml of medium for 3 h. Enzymes and/or proteinase inhibitors [20 μg of ovomucoid and 10% (v/v) newborn-calf serum] were then added in 125 μl of medium, and the samples were incubated for an additional 1 h before the determination of the amount of cell-attached ^{35}S -labelled proteoglycan. The values given are the means of two determinations. Abbreviation: TRU, turbidity-reducing unit (Ohya & Kaneko, 1970).

Addition	^{35}S radioactivity bound to cells (d.p.m.)
Medium	27 150
Medium with proteinase inhibitors	24 357
Medium with 1 TRU of <i>Streptomyces</i> hyaluronidase + proteinase inhibitors	1060
Medium with 0.1 TRU of <i>Streptomyces</i> hyaluronidase + proteinase inhibitors	1492
Medium with 0.01 TRU of <i>Streptomyces</i> hyaluronidase + proteinase inhibitors	2139
Medium with 100 ng of trypsin	4963
Medium with 10 ng of trypsin	26 009
Medium with 1 ng of trypsin	24 770

Fig. 6. Reversibility of binding of [^{35}S]sulphate-labelled proteoglycans to chondrocytes

^{35}S -labelled proteoglycans ($5\mu\text{g}$ in $10\mu\text{l}$ of stock solution) were added to the cell suspension. After 1 h or 4 h of incubation the cultures were centrifuged. The medium was withdrawn and the cells were resuspended in fresh medium with or without competitors. Concentrations of competitors were chosen to give about 80% inhibition if added with the labelled proteoglycan (cf. Fig. 5). Incubations were terminated as described in the Experimental section. ●, Control without competitors; □, articular cartilage monomer ($256\mu\text{g}$); ■, hyaluronic acid-binding region ($4\mu\text{g}$); △, hyaluronic acid dodecasaccharide ($64\mu\text{g}$); ▲, hyaluronic acid ($16\mu\text{g}$).

formed in the medium of chondrocyte cultures and present in cartilage matrix. Internalization is not a major process. Therefore, during the time period studied, the added ^{35}S -labelled proteoglycans seem to be integrated as a part of the pericellular matrix. The proteoglycan molecules in this matrix appeared to be bound to the cells by specific bonds, since reduced and alkylated ^{35}S -labelled proteoglycans showed no binding.

The different binding of proteoglycans isolated from cultured chondrocytes and isolated from cartilage is puzzling in view of the fact that both preparations have equal capacity to bind to hyaluronic acid *in vitro*. It may be that the preparations contain different proportions of the two subpopulations of aggregating proteoglycans (Heinegård *et al.*, 1981), or that proteoglycans are modified with time in the extracellular matrix, such that their hyaluronic acid-binding regions bind less strongly, either to hyaluronic acid or to stabilizing factors.

In cartilage chondrocytes are embedded in an

extensive extracellular matrix, containing predominantly collagen and proteoglycans. The chondrocytes seem to be able to regulate the content of macromolecules in this matrix (Hardingham *et al.*, 1972), by an unknown mechanism. It is possible that feedback control by an interaction between proteoglycans in the matrix and a cell-surface receptor for proteoglycans play a role (Nevo & Dorfman, 1972; Wiebkin & Muir, 1977; Lowther & Handley, 1979). Other potential regulating factors are hormonal influences, mechanical properties of the matrix, and interactions with collagen and other matrix components. Suspension cultures are well suited for identification of specific cell-surface receptors for proteoglycans, as the cells do not accumulate an extracellular matrix as in monolayer cultures. The approach used does not exclude additional mechanisms for proteoglycan association with the chondrocyte. There may be a pool of proteoglycans bound to the cell surface by a different mechanism.

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