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Structural colocalisation of type VI collagen and fibronectin in agarose cultured chondrocytes and isolated chondrons extracted from adult canine tibial cartilage

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ABSTRACT

Cell–matrix and matrix–matrix interactions are of critical importance in regulating the development, maintenance and repair of articular cartilage. In this study, we examined the structural colocalisation of type VI collagen and fibronectin in isolated chondrons and long-term agarose cultured chondrocytes extracted from normal adult canine articular cartilage. Using double labelling immunohistochemistry in conjunction with dual channel confocal microscopy and digital image processing we demonstrate that type VI collagen and fibronectin are distributed in a similar staining pattern and are colocalised at the surface of cultured chondrocytes and isolated chondrons. The results suggest that type VI collagen and fibronectin may play a role in both cell–matrix adhesion and matrix–matrix cohesion in the pericellular microenvironment surrounding articular cartilage chondrocytes.

Key words: Articular cartilage; dog; extracellular matrix; matrix–matrix; confocal microscopy.

INTRODUCTION

The interaction between the chondrocyte and its extracellular matrix is of critical importance in regulating the development, maintenance and repair of articular cartilage. It is generally accepted that the cells in adult articular cartilage are surrounded by a specialised pericellular microenvironment; the chondrocyte, its pericellular matrix and capsule represent the chondron, a natural microanatomical unit of adult articular cartilage (Poole et al. 1987, 1988*a*). Immunohistochemical studies have now characterised the heterogeneous composition of the pericellular microenvironment which contains collagen types II, VI, IX (Poole et al. 1988*b*, 1988*c*) and XI (Smith et al. 1989), the proteoglycans aggrecan (Poole et al. 1990, 1991) and decorin (Poole et al. 1993), and glycoproteins such as fibronectin (Poole 1990) and link protein (Ratcliffe et al. 1984). In particular, type VI collagen has been identified as an important matrix component in a range of connective tissues (Timpl & Engel, 1987), and has emerged as a discrete molecular marker of chondron microanatomy, interacting both with the cell surface and with the heterogeneous components of the pericellular microenvironment (Poole et al. 1988*b*, 1992).

Recent biochemical studies have shown that many extracellular macromolecules interact. The interactions of particular relevance to articular cartilage include those between aggrecan, link protein and hyaluronan (Hardingham et al. 1992; Heinegård & Pimentel 1992), and between collagen types II, IX and XI (Eikenberry et al. 1992). It has also been reported that type VI collagen interacts with a range of matrix macromolecules including type II collagen, decorin and fibromodulin (Bidanset et al. 1992), hyaluronan (McDevitt et al. 1991; Kielty et al. 1992), and fibronectin (McDevitt & Marcelino, 1993). Of these macromolecules, fibronectin has been identified as a ubiquitous multifunctional glycoprotein participating in a wide variety of cellular activities including differentiation, adhesion, and migration (Hynes, 1990; Yamada, 1991). These activities result from the ability of the disulphide linked fibronectin dimers to interact

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with a number of biological structures including the cell surface receptors, a range of collagen species, fibrin and heparin. Thus fibronectin primarily functions as a connecting molecule in the extracellular matrix, mediating both cell–matrix and matrix–matrix interactions (Hynes, 1990; Yamada, 1991).

Since both type VI collagen and fibronectin have been reported in the pericellular region surrounding articular cartilage chondrocytes (Evans et al. 1983; Poole et al. 1988*b*, 1992), and their synthesis is upregulated in osteoarthritis (McDevitt et al. 1988; Ronzière et al. 1990), we considered it important to establish the spatial relationship and the potential for structural interactions between type VI collagen, fibronectin and the chondrocytes in mature articular cartilage. Previous studies have demonstrated the colocalisation of these 2 matrix macromolecules in placenta (Amenta et al. 1986), neoplasms (McComb et al. 1987), and meniscus (Futani & McDevitt, 1995). In addition, Wolf & Carsons (1991) have demonstrated differences in the distribution of type VI collagen in normal and rheumatoid synovial tissue and cultured synoviocytes with relation to fibronectin expression, suggesting that type VI collagen may play a part in remodelling of the inflamed synovium. More recently, confocal laser scanning microscopy and digital image processing have been used to show decreased matrix fibronectin and type VI collagen between overlapping human rheumatoid synoviocytes cultured in the presence of added fibronectin, suggesting both macromolecules may be metabolically and structurally coordinated (Wang et al. 1995). Although microscopic analysis of isolated canine chondrons and cultured chondrocytes has detailed the pericellular localisation of type VI collagen (Poole et al 1988*b*, 1992, Chang & Poole, 1996) and to a lesser extent, fibronectin (Poole 1990), there are currently no reports on the relative spatial distribution of these 2 matrix macromolecules either in articular cartilage chondrons or phenotypically stable chondrocytes maintained in suspension culture.

In the present study, double label immunohistochemistry in conjunction with dual channel confocal microscopy and digital image processing were used to examine the structural colocalisation of type VI collagen and fibronectin in isolated chondrons and long-term agarose cultured chondrocytes extracted from normal adult canine articular cartilage. The results demonstrate that type VI collagen and fibronectin colocalise at the surface of cultured chondrocytes and isolated chondrons where they are thought to play a role in both cell–matrix adhesion and matrix–matrix cohesion during the development and maintenance of the pericellular microenvironment.

MATERIALS AND METHODS

Materials

Hams F-12 culture medium, fetal bovine serum (FBS) and bovine serum albumin (BSA) were obtained from GIBCO, Life Technologies, New Zealand, sea plaque agarose from FMC Bioproducts, USA, pronase from Boehringer Mannheim, NZ, and collagenase and testicular hyaluronidase from Sigma Chemical Co., USA. CellTracker Green (5-chloromethylfluorescein diacetate; CMFDA) and ethidium homodimer-1 were obtained from Molecular Probes, Oregon, USA. Anti-type VI collagen antibody was kindly donated by Dr Shirley Ayad, University of Manchester. Antifibronectin antibody was purchased from GIBCO, Life Technologies, New Zealand.

Tissue sources

Fresh cartilage samples were collected through the full depth of the tibial plateaux and femoral condyles of mature, healthy crossbred dogs (2–4 y) euthanised under veterinary supervision at the Auckland City Pound.

Isolation of chondrocytes

Tissue collected from both the tibial plateaux and femoral condyles was pooled and chondrocytes isolated as previously described (Aydelotte & Kuettner, 1988). Briefly, diced cartilage was treated with pronase (0.8% w/v, at 37 °C for 90 min) in Ham's F-12 medium containing 5% FBS, followed by bacterial collagenase digestion (0.4% w/v) at 37 °C for 19 h. Released chondrocytes were collected by centrifugation, washed in medium, and total cell number determined by haemocytometer counting. Cells were suspended in a solution of 1% (w/v) agarose gel in Ham's F-12 medium with 10% FBS, plated at a density of 1×10^6 cells/35 mm dish, and maintained in culture for up to 12 wk.

Triplicate cultures were sampled at selected time points (1 d, 1 wk, 6 wk, 12 wk); 1 culture was immediately treated with 25 μM/ml CMFDA at 37 °C for 2 h to load viable cells (Poole et al. 1996), and all 3 cultures were fixed in 4% paraformaldehyde for 30 min and washed extensively. Chondrocyte-agarose plugs were cored from the centre of the culture dish with a core punch (6 mm diameter) and stored in phosphate-buffered saline (PBS) supplemented with

1% BSA and 0.05% Na azide at 4 °C as previously described (Poole et al. 1991).

Isolation of chondrons

Chondrons were extracted from tibial cartilage samples as previously described (Poole, 1990). Briefly, the diced cartilage sample was serially homogenised in 20 ml of PBS at relatively slow speeds for short periods, and the flocculent supernatant collected. Pooled supernatants were filtered, collected by centrifugation and washed in PBS at 4 °C. The final prepared chondron homogenate was suspended in a solution of 1.5% (w/v) agarose gel in Ham's F-12 medium with 10% FBS, and cast directly into 35 mm culture dishes to create a chondron-agarose monolayer 0.5–0.8 mm thick. Prior to fixation, several chondron agarose preparations were treated with 25 μM/ml CMFDA at 37 °C for 4 h to visualise viable chondrocytes in the preparation (Poole et al. 1996).

Both CMFDA loaded and unloaded preparations were fixed with 4% paraformaldehyde for 30 min, washed extensively, and chondron-agarose plugs cored from the centre of the culture dish as described above.

Immunohistochemistry

Two experimental subsets were established for immunohistochemistry. (1) To examine the relationship between the chondrocyte and its pericellular matrix, CMFDA was used to produce a fluorescent green profile of viable chondrocytes while Texas red was used to contrast the individual distribution of either type VI collagen or fibronectin at the cell surface. (2) To examine the relationship between pericellular matrix components, non-CMFDA loaded preparations were used allowing fibronectin to be assigned the FITC fluoroprobe, and type VI collagen the Texas red fluoroprobe.

Both chondrocyte-agarose plugs and chondron-agarose plugs were pretreated with testicular hyaluronidase (2 mg/ml in 0.1 M Tris saline, pH 5.5) to remove matrix proteoglycans which otherwise mask antigenic sites on both the type VI collagen and fibronectin macromolecules (Poole et al. 1991, 1992). Chondrocyte-agarose plugs were digested at room temperature for 60 min, while chondron-agarose plugs were treated at 37 °C for 2 h followed by overnight digestion (18 h) at room temperature. Anti-type VI collagen antibody was prepared at a dilution of 1:500 in 0.1 M Tris saline with 1% BSA, and the plugs labelled for 24–48 h at 4 °C with continuous agitation. A biotinylated donkey antirabbit antibody (1:100) and a streptavidin linked Texas red fluoroprobe (1:50) were used for detection of the antitype VI collagen antibody. Plugs were washed extensively (3×60 min) to completely remove unbound antibodies, and lightly fixed to immobilise bound antibodies. The staining regime was then repeated using the antifibronectin antibody (1:500–1:1000), and the fibronectin distribution visualised using an FITC-linked donkey antirabbit secondary antibody (1:100). In this way, the possibility of cross-reactivity between antibodies and fluoroprobes was minimised. Treatment with normal rabbit serum was used as a negative control, while cryosections of human placenta were used as positive controls. Immunolabelled plugs were finally mounted in Vectashield (Vector Laboratories, California, USA) and the margins of the coverslip sealed with clear nail varnish.

Confocal microscopy

Confocal images were obtained using a Leica TCS 4d Confocal Laser Scanning Microscope (Leica, Heidelberg, Germany) equipped with a Krypton-Argon laser source, Leica 63X (NA = 1.4) and 100X (NA = 1.3) infinity adjusted oil immersion objectives and dual channel photodetectors. By precisely stepping the microscope stage in the vertical z-axis, a series of thin (approximately 0.5 μm), perfectly focused optical sections were collected at different x-y planes through the specimen.

The confocal system was operated in fluorescence mode to image the distribution of Texas red labelled type VI collagen in the section, the maximum and minimum pixel intensities being optimised for the Texas red photomultiplier channel. To image the distribution of CMFDA and fibronectin a specific FITC filter set was selected, the voltage and offset of the photomultiplier channel being adjusted to optimise pixel intensity. For direct correlation studies, 10–15 optical sections, approximately 1 μm apart, were collected at an excitation wavelength of 488 nm to image the distribution of either CMFDA or fibronectin. The 568 nm line of the laser was then selected to excite the Texas red fluoroprobe, and a separate data set of type VI collagen collected through the identical z-axis coordinates. The opticodigital data sets collected during each scan were transferred to a Silicon Graphics IRIS Indigo R4000 workstation and permanently stored on rewritable magneto-optical disks.

Each data set collected on the confocal microscope was processed using the 3D software included with the

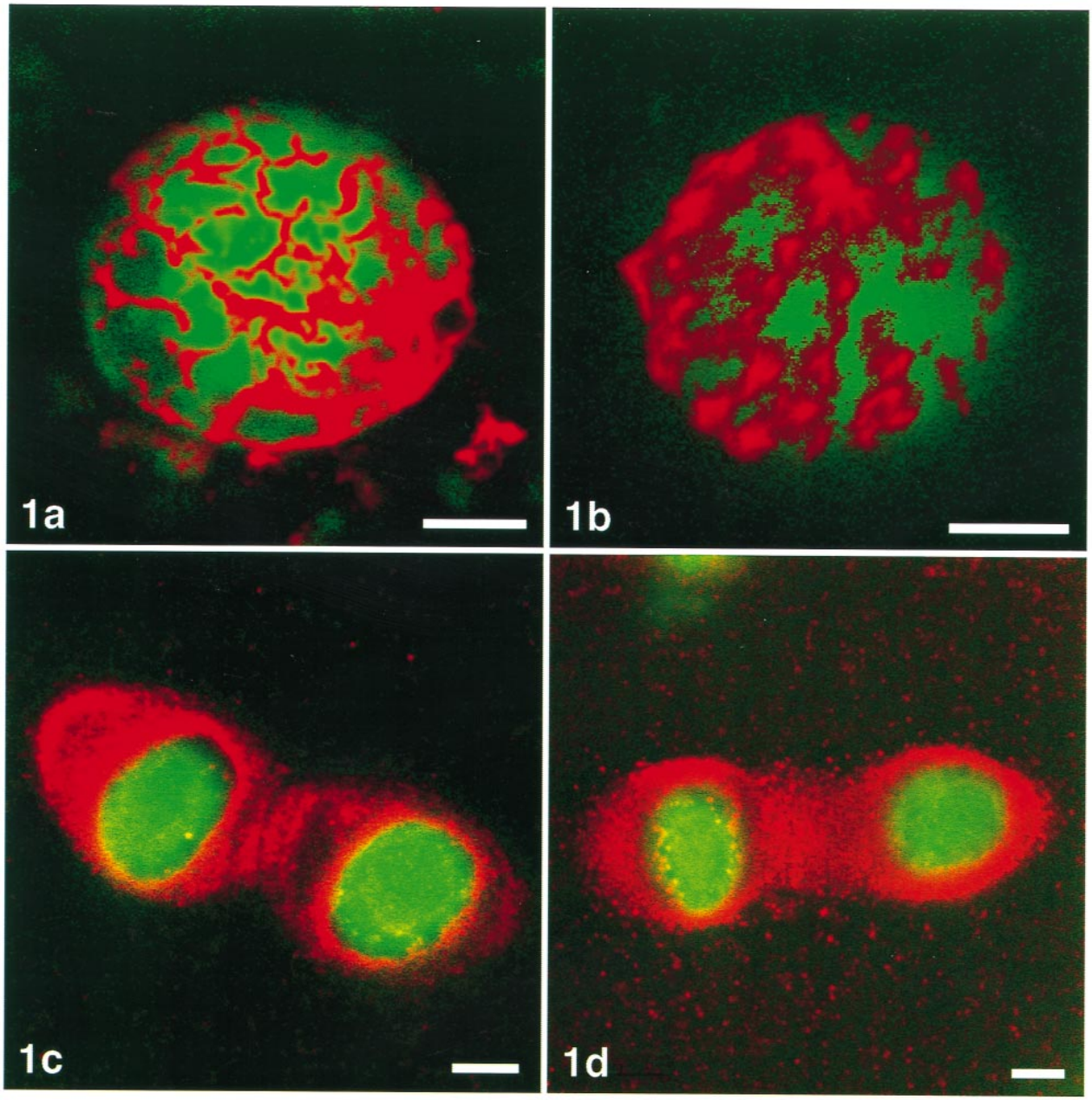

Fig. 1. Digital confocal images of cultured chondrocytes (*a*, *b*) and isolated chondrons (*c*, *d*) labelled with CMFDA and either anti-type VI collagen (*a*, *c*) or antifibronectin antibodies (*b*, *d*). After 2 wk in culture, chondrocytes loaded with CMFDA (green) were surrounded by a 'lattice-like' pattern of both type VI collagen (*a*) and fibronectin labelling (*b*). Extended focus projection showed type VI collagen staining was consistently strong and uniform (*a*) while fibronectin labelling appeared stippled, and less consistent (*b*). Single optical sections of isolated chondrons showed similar distribution patterns, although fibronectin staining was stronger and more stippled in the interconnecting segments between adjacent chondrons (*c*, *d*). Bars, 5 μm.

Leica ScanWare operating system to construct a z-series projection. This results in a perfectly focused image through the depth of the specimen, and was saved in TIFF file format before transfer to a Power Macintosh 7100/80 AV for digital image processing.

Digital image processing

Digital images showing cell–matrix and matrix–matrix interactions were processed and assembled using Adobe Photoshop 4.0 (Adobe Systems, USA). To

but a lack of colocalisation in the further removed matrix (arrowhead). Single optical section through isolated chondrons showed type VI collagen concentrated around the chondrocyte with a weaker reaction in the interconnecting segments between adjacent chondrons (*g*). Conversely, fibronectin appeared weaker in the capsule and stronger in the interconnecting segments (*h*; arrowhead), but colocalised precisely with type VI collagen (*i*). Representative reconstructions showed the consistency of this colocalisation in isolated chondrons (*j–l*), although fibronectin showed a broader matrix distribution than type VI collagen (*j*). Bars, 10 μm.

[*Legend continues at foot of facing page*

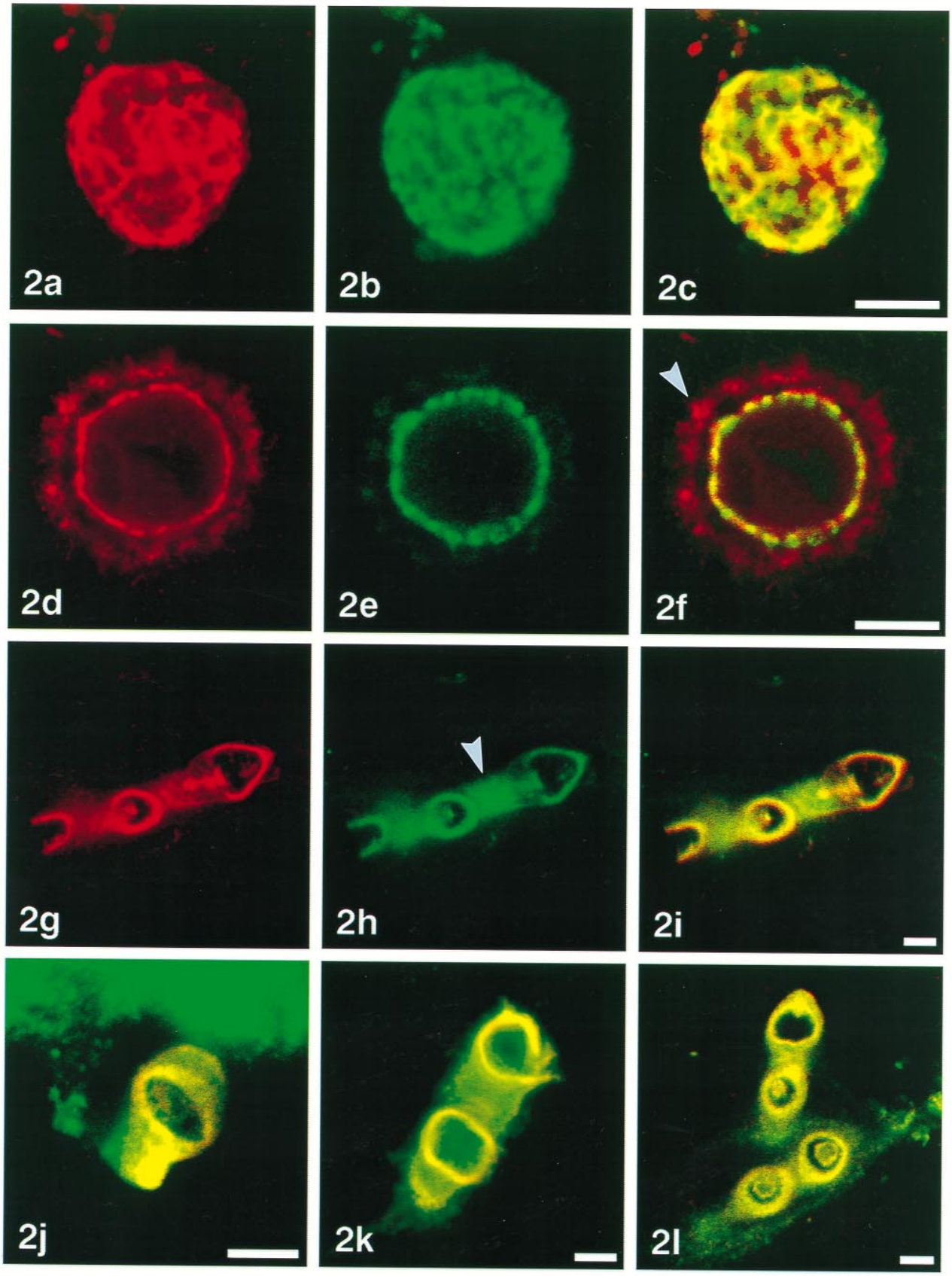

Fig. 2. Colocalisation of type VI collagen and fibronectin in cultured chondrocytes (*a-f*) and isolated chondrons (*g–l*). After 6 wk in culture (*a-c*), extended focus reconstructions of type VI collagen (*a*) and fibronectin (*b*) show both molecules structurally colocalised when the images were merged (*c*). After 12 wk in culture, single optical sections revealed more extensive type VI collagen labelling (*d*) when compared with fibronectin in the same preparation (*e*). Digital reconstruction revealed precise colocalisation of both macromolecules at the cell surface (2f),

demonstrate the relationship between the chondrocyte and its pericellular microenvironment, an extended focus image of all FITC sections through a CMFDA loaded cell was first prepared and assigned a bright green colour from the colour-look-up table. For cultured chondrocytes, one half of the Texas red data set, representing a hemispheric view of either type VI collagen or fibronectin distribution, was prepared in extended focus and assigned a bright red colour. In contrast, the density of the capsule in isolated chondrons obscured cell morphology precluding a similar hemispheric view, and for these preparations a optical section from the middle of the Texas red data set was prepared and assigned a bright red colour. The 2 images were then overlaid using Adobe Photoshop commands to produce both 'exterior' and 'cutaway' views of the cell–matrix relationship in cultured chondrocytes and isolated chondrons respectively.

To demonstrate the relationship between pericellular matrix components, both extended focus images of half the data set and single optical sections through the middle of the chondrocyte were prepared using the Leica Scanware software. The FITC labelled distribution of fibronectin and the Texas red labelled distribution of type VI collagen were combined using Adobe Photoshop and the resulting yellow-orange colours used to identify the colocalisation between these 2 macromolecules.

RESULTS

Cell–matrix relationships

Cultured chondrocytes. The relationship between viable chondrocytes and type VI collagen or fibronectin during the formation of the pericellular microenvironment in vitro are shown in Figure 1*a* and 1*b* respectively. At 2 wk, reconstructed images showed that both type VI collagen and fibronectin have a 'lattice-like' distribution stretched over the surface of somewhat swollen chondrocytes typical of these cultures (Fig. 1*a*,*b*). The staining patterns of these 2 macromolecules are very similar. However, type VI collagen staining appeared more discrete and uniformly distributed throughout the structure (Fig. 1*a*), while fibronectin staining was more variable, with weaker staining in the rods of the lattice and stronger, more punctate staining at the nodes (Fig. 1*b*).

Isolated chondrons. The relationship between viable chondrocytes and the pericellular microenvironment in isolated chondrons labelled individually with either anti-type VI collagen or antifibronectin antibodies are shown in Figure 1*c* and 1*d* respectively. Double labelled reconstructions of type VI collagen showed intense, uniform staining in the pericellular matrix and capsule surrounding CMFDA loaded chondrocytes, with weak stippled staining in the tail and interconnecting segments between adjacent chondrons (Fig. 1*c*).

Contrasting reconstructions of viable chondrons labelled with antifibronectin antibody showed a similar distribution pattern to type VI collagen, although fibronectin is more concentrated in the interconnecting segments between adjacent chondrons (Fig. 1*d*). Like the cultured chondrocytes, fibronectin staining was less uniform and more stippled than type VI collagen, but often extended into the remnants of extracellular matrix typically adherent to mechanically isolated chondrons (see Fig. 2).

Matrix–matrix relationships

Cultured chondrocytes. The colocalisation of type VI collagen and fibronectin in agarose cultured chondrocytes is shown in Figure 2*a-f*. When imaged separately as extended focus images, both type VI collagen (Fig. 2*a*) and fibronectin (Fig. 2*b*) showed a virtually identical 'lattice-like' distribution after 6 wk in culture. Again fibronectin staining was less consistent than type VI collagen (Fig. 2*b*). The combination of red and green images produces a bright yellow colour indicating a highly specific colocalisation of type VI collagen and fibronectin (Fig. 2*c*). Orange/yellow and yellow/green colours result from variable quantities of each macromolecule at precise points on the cell surface. A similar, but less developed pattern of colocalisation was evident after 1 wk culture (results not shown), and was most clearly evident by 12 wk (Fig. 2*d-f*). When imaged separately as single optical sections, type VI collagen (Fig. 2*d*) and fibronectin (Fig. 2*e*) showed a similar distribution at the cell surface, while a second, less distinct layer of type VI collagen was often observed beyond from the pericellular matrix. Combination of these images confirmed the high degree of colocalisation between type VI collagen and fibronectin at the cell surface, but not with the type VI collagen further removed from the cell (Fig. 2*f*). Reconstructions from cells after 1 and 6 wk in culture showed similar patterns of colocalisation (results not shown).

Isolated chondrons. The colocalisation of type VI collagen and fibronectin in isolated chondrons is shown in Figure 2*g–l*. When imaged separately as single optical sections, type VI collagen (Fig. 2*g*) and

fibronectin (Fig. 2*h*) showed similar, yet subtly different patterns of distribution in the same optical plane. Type VI collagen was concentrated around the chondrocyte with weaker staining in the interconnecting segments between adjacent chondrons in the column (Fig. 2*g*). Similarly, fibronectin staining was concentrated in the pericellular capsule and matrix around each chondrocyte, but in contrast to type VI collagen, remained relatively strong in the tail and interconnecting segments (Fig. 2*h*). Digital reconstructions confirmed the structural colocalisation of both macromolecules in isolated chondrons (Fig. 2*i*), variations in their relative concentrations presenting as orange/yellow and yellow/green regions within the microstructure of the pericellular microenvironment. Both macromolecules colocalised in the pericellular matrix and capsule, while fibronectin was more prominent in the tail and interconnecting segments, and had a broader matrix distribution than type VI collagen (Fig. 2*j*). Several reconstructions combining type VI collagen and fibronectin images (Fig. 2*j-l*) confirmed the structural colocalisation of these macromolecules as a general feature of chondron microanatomy.

DISCUSSION

This is the first study to demonstrate the structural colocalisation of type VI collagen and fibronectin in the pericellular microenvironment of mature articular cartilage chondrocytes. The combined use of vital dyes and immunohistochemical techniques, coupled with dual channel confocal microscopy and digital image processing also indicate that suspension cultured chondrocytes sequester type VI collagen and fibronectin in the pericellular microenvironment, and that both macromolecules were structurally localised at identical points on the chondrocyte surface throughout extended culture periods. Type VI collagen and fibronectin were also colocalised in the pericellular matrix and capsule of isolated chondrons, with higher levels of fibronectin in the tail and interconnecting segments between adjacent chondrons. However, in contrast to type VI collagen, fibronectin was more broadly distributed both in the territorial and interterritorial matrices, confirming earlier observations which indicate that type VI collagen is a discrete and reliable marker of the pericellular microenvironment in intact articular cartilage (Ayad et al. 1984; Poole et al. 1992), in suspension labelled chondrons (Poole et al. 1988*b*, 1992), and the chondrocytes and osteocytes of the intervertebral disc (Roberts et al. 1991). Together these data imply a degree of coordinated production and sequestration of type VI collagen and fibronectin in vitro, and point to a role for cell surface receptors in the development and assembly of the pericellular microenvironment. Moreover, the maintenance of this colocalisation in mature chondrons implies a continued role for type VI collagen and fibronectin in the structural integrity of chondrons in adult articular cartilage.

Type VI collagen is a hybrid molecule composed of a short triple helix with large N- and C-terminal glycoproteins (Engel et al. 1985; Kielty et al. 1990) containing several repeating motifs which facilitate the diverse adhesive functions attributed to this collagen species (Keene et al. 1988; McDevitt et al. 1991; Bidanset et al. 1992; Doane et al. 1992; Kielty et al. 1992; Specks et al. 1992; McDevitt & Marcelino, 1993). Similarly fibronectin is a multifunctional glycoprotein with multiple binding sites for extracellular macromolecules (Glant et al. 1985; Hynes, 1987; Ruoslahti, 1988; Akiyama et al. 1990; Schmidt et al. 1991; Chevalier, 1993; McDevitt & Marcelino, 1993), and represents a well established feature of the cell–matrix adhesion complex (Hynes, 1990; Yamada, 1991). It has also been established that many of the matrix components which interact with type VI collagen and fibronectin will interact with a range of additional matrix macromolecules. Thus collagen types II, IX and XI in the pericellular microenvironment form fine heterotypic fibrils (Eikenberry et al. 1992) in which the cationic NC4 domain of type IX collagen projects from the fibril surface to interact with the anionic proteoglycans in the extracellular matrix (Smith & Brandt, 1992). Alternatively, hyaluronan binds with link protein and the protein core of aggrecan to form large aggregates which provide the hydraulic load-bearing capacity of the articular cartilage matrix. Decorin on the other hand is thought to regulate fibril assembly and is known to bind TGF-β in the matrix (Yamaguchi et al. 1990; Streuli et al. 1993), suggesting a function in metabolic regulation in the vicinity of the chondrocyte. Thus in addition to providing adhesion between the chondrocyte and its pericellular microenvironment, type VI collagen and fibronectin could promote macromolecular cohesion between matrix components critical for the development and maintenance of the chondron.

The mechanism by which newly synthesised type VI collagen and fibronectin were retained in the pericellular microenvironment was not examined in this study. However, published reports indicate that the interaction between the chondrocyte and these macromolecules is potentially mediated by a range of cell

surface receptors, notably the integrin receptors a1b1 and $\alpha 2\beta 1$ which are specific for type VI collagen (Salter et al. 1995), and $\alpha 5\beta 1$ specific for fibronectin (Salter et al. 1992; Shakibaei et al. 1993). Recent studies (Hirsch et al. 1996) reporting a punctate distribution of $\alpha 2$, $\alpha 3$ and $\beta 1$ integrin subunits on the surface of cultured chondrocytes are also consistent with data presented showing a stippled, lattice-like distribution of type VI collagen and fibronectin close to the cell surface, and further support the suggestion that integrin receptors may play a role in assembly of the pericellular microenvironment. In addition, a range of nonintegrin receptors such as anchorin CII (annexin V) (Kirsch & Pfaffle, 1992), the hyaluronan receptor CD44 (Bidanset et al. 1992; Kielty et al. 1992; Knudson, 1993) and the developmental receptor NG2 (Stallcup et al. 1990) may also be involved in pericellular matrix assembly through their specificity for macromolecules known to interact with both type VI collagen and fibronectin. The patterns of macromolecular sequestration in long term agarose cultures were particularly interesting in this regard since they were thought to mirror the developmental assembly of the pericellular microenvironment. Thus in the early stages of development, both type VI collagen and fibronectin were specifically colocalised in a lattice-like pattern which enveloped the chondrocyte. However, the nature of the pericellular macromolecules which occupy the spaces within the lattice have yet to be determined, but could represent hyaluronan and/or aggrecan, identified in parallel studies (Chang & Poole, personal observations), and previously shown to be involved in the in vitro assembly of the pericellular microenvironment (Knudson, 1993; Lee et al. 1993). By the end of the 12 wk culture period, the entire chondrocyte was enclosed by an intimate glycocalyx of type VI collagen and fibronectin, a situation which persists in the chondrons of mature articular cartilage. These observation have led us to speculate that type VI collagen and fibronectin could act cooperatively to anchor a collagen and glycoprotein rich calyx at the cell surface, and could subsequently act as molecular scaffolds for the continued assembly and differentiation of matrix macromolecules essential for the supramolecular architecture and functional stability of the pericellular microenvironment surrounding mature articular cartilage chondrocytes.

In summary, this study demonstrates that type VI collagen and fibronectin colocalise in the pericellular microenvironment of agarose cultured chondrocytes and isolated chondrons derived from normal adult canine articular cartilage. The results suggest that type VI collagen and fibronectin act synchronously to coordinate cell–matrix and matrix–matrix interactions during the development and maintenance of the pericellular microenvironment surrounding articular cartilage chondrocytes.

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