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ISOLATED CHONDRONS: A CONCEPT COMES OF AGE!

C. Anthony Poole
Department of Anatomy, University of Auckland Medical School, New Zealand.

ABSTRACT

• *The chondron concept was first introduced in 1925 by Benninghoff who used polarised light microscopy to show that the chondrocyte in hyaline cartilages was surrounded by a specialised region of matrix. However, this concept was not widely accepted a (the time, audit was until 1969 that Szirmai rekindled interest in the chondron concept. Using new histochemical interpretations coupled with rnicrochernical analysis, Szirmaishowed the "micro-world"'of thechondron consisted of a fine collagenous ' 'perilacunar rim " surrounding aproteogycanrich ' lacunarspace". He also showed that chondrons could be extracted from horse nasal septum by high speed homogenisation and concluded that the chondron was mechanically robust and represented the primary functional and metabolic unit of hayline cartilages. Again the chondron concept was not widely embraced despite the rapid accumulation of data indicating common specialisations in the vicinity of hayline cartilage chondrocytes. In 1985, our own research on thestnicture and function of adult articular cartilage matrices led to the discovery of chondrons in the homogenate produced by slow speed homogenisation of canine tibial cartilage. The isolation of intact chondrons conclusively established the chondron as a microanatomical unit of adult articular car-tilage. Each chondron consisted of a chondrocyte intimately associated with a proteoglycan rich pericellular matrix which was surrounded by a densely compacted pericellular capsule composed of fine collagen species. We have now developed the isolated chondron as a model to study the natural interrelationship between the chon-drocyte and its pericellular microenvironment. Using morphological, histochemical, ultrastnictural, immunohistochemical, biochemical and metabolic techniques, we have successfully shown the isolated chondron is rich in chondroitin sulphate, keratan sulphate and hyaluronan glycosaminoglycans, collagen types II, VI, IX, and XI, and glycoproteins such as fibronectin and possibily laminin. Further studies of chondrons extracted from osteoarthritic cartilage suggest the chondron is remodelled during the degenerative process causing the pericellular microenvironment to expand and the chondrocyte to proliferate into the large clusters typical of osteoarthritic pathology. The data presented in this short review indicates that the chondron is a highly specialised microanatomical unit of adult articular cartilage and plays a fundamental role in the initiation and progression of degenerative arthritis. Future studies on the isolated chondron model will ensure a highly greater understanding of the role of the cellular microenvironment in articular cartilage biology and pathology.*

EVOLUTION OF THE CHOMDROH COHCEPT

• The concept of the chondron was first introduced by Benninghoff (1) to describe the chondrocyte and its specialised microenvironment in a range of hyaline cartilages including adult articular cartilage. Each chondron was shown to consist of a chondrocyte,its 'lacunar space" and "perilacunar rim", and it was proposed that they represent the primary functional and metabolic unit responsible for cartilage matrix homeostasis (1,2). Some authors supported the concept of the chondron as a compression resistant, water filled "bladder" (3) but in general, the lack of correlation between Benninghoff's polarising microscope data and the accepted histochemical dogma of the period lead to the general dismissal of the chondron concept (4).

In the 1960's however, Szirmai used new histochemical concepts and techniques in conjunction with microchemical analysis to demonstrate the heterogeneous distribution of proteoglycans and collagen in the matrix of

horse nasal cartilage (2). He also suggested that chondrons were extremely mechanically robust structures, citing as evidence the presence of intact chondrons in the cartilage-bone fracture plane of an accident victum (4) and in the sediment resulting from high speed homogenisation of horse nasal septum (2). The sum of these results prompted Szirmai to conclude that the chondron could be described as a separate anatomical, mechanical and physiological unit of cartilage (2,4). Yet despite Szirmai's excellent studies, little attention has been given to the subject since his retirement in the early 1970's (personal communication). Our discovery, in 1985, that intact chondrons could be extracted from adult canine tibial cartilage by slow speed homogenisation techniques has completely vindicated the conclusions of Benninghoff and Szirmai. This review briefly outlines the organisation of the pericellular microenvironment in adult articular cartilage and the development of the isolated chondron as a model to study cell-matrix interactions in articular cartilage health and disease,

CHONDRONS: THE CHONDROCYTE AND ITS PERICELLULAR MICROEHVIRONMEMT IM HYALINE CARTILAGES

• Histochemical techniques at both the light and electron microscopic levels have played a major role in defining cartilage matrix heterogeneity (2,5,6). These studies showed a progressive increase in the concentration of chondroitin sulphate and keratan sulphate from the superficial to the deep layers of the cartilage (2), and a clear subdivision of the middle and deep layers into pericellular, territorial and interterritorial matrices (5). Alcian blue staining at critical electrolyte concentrations (6) has been particularly important in developing our understanding of the differential glycosaminoglycan distribution inherent in the articular cartilage matrix. Using this method, keratan sulphate was found to be more concentrated in the interterritorial matrix and increase with ageing, chondroitin sulphate was more concentrated in the territorial matrix, but decreases with ageing, while the pericellular matrix contains chondroitin sulphate (2) and a significant proportion of the total matrix hyaluronic acid (hyaluronan) (6,7).

Ultrastructural studies using cationic electron dense stains such as ruthenium red and ruthenium hexamine trichloride (8,0) have confirmed the high concentration of proteogycans in the pericellular microenvironment. This intense reaction could be reduced or eliminated by digestion with bacterial hyaluronidase, chondroitinase ABC and AC, or 4M guanidinium hydrochloride extraction (8). Interestingly, a small proportion of the matrix proteoglycans (10-20%) were found to be resistant to this latter extraction technique (8,10,11) and could be histochemically localised in the pericellular microenvironment. Collagenase extraction and analysis of these resistant proteoglycans showed significant amounts of hydroxyproline in the fraction, suggesting that aportion of the resistant proteoglycans were firmly bound to collagen (11). Subsequently, a chondroitin sulphate chain was identified which is covalently linked to the non-collagenous NC3 domain of more than 70% of the ct2 IX polypeptide chains of type IX collagen isolated from hyaline cartilage (12,13). This demonstrated for the first time an identifiable link between the collagen and proteoglycan components of hyaline cartilages.

Collagen distribution in the extracellular matrix is more difficult to assess histochemically. Here, interference contrast techniques have been important in demonstrating the specialised organisation of finely woven fibrils which form an anisotropic band or "halo" around the cell and its pericellular matrix (7,14). A number of ultrastructural investigations of collagen organisation in articular cartilage have been published (5,15) and are consistent in showing fine fibrils in the vicinity of the cell, and a progressive increase in fibre size through the territorial and interterritorial matrices. Scanning electron microscopy has confirmed the tight packing of fine collagen fibres around the chondrocyte and interdigitated anmongst the coarser fibres of the adjacent territorial matrix (16).

Several new minor collagens have now been identified in hyaline cartilages and represent 5-10% of the total collagenous protein (17). Type IX has been most extensively studied (12,13,18-20) and recent evidence has shown it is crosslinked via hydroxypyridinium bonds to type II collagen is decorated in a "bottle-brush" fashion by the type IX collagen molecules which project from its surface (21). The functiuon of this interaction is unknown, although it could serve to control lateral growth of type II collagen fibrils in areas rich in type IX collagen (22,23). Type VI collagen has also been identified in articular cartilage (17) and is enriched in the early stages of experimentally induced canine osteoarthritis (24). The function of type VI collagen in cartilage is entirely unknown but it has been implicated in cell-matrix adhesion and proteoglycan interaction (24), as an anchoring network involved in stabilisation of the major collagen species in the matrix (25), and has recently been shown to interact with a membrane intercalated chondroitin sulphate proteoglycan called NG2 (26). Finally, type XI (la, 2a, 3o) collagen (27,28) has also been immunolocalised in articular cartilage (29) and identified in the pericellular coat surrounding suspension cultured chodrocytes (27). While the function of this collagen remains obscure, recent evidence which shows that type XI collagen is associated with the chondrocyte cell surface (28) and interacts with pericellular proteoglycans (28) suggests type XI collagen may play a role in pericellular matrix formation and organisation. Indeed, the more recent studies from this rapidly

developing field indicate that embryonic chick cartilage contains mixed fibrils of types II, IX, and XI collagens (30) further compounding our interpretation of the functional interdependency of the major and minor collagen species in the matrix of adult articular cartilage.

The introduction and application of immunohistochemical techniques to the study of the extracellular matrix has significantly advanced our understanding of the heterogenous nature of cartilage components. Numerous studies have now reported on the light and electron microscopic distribution of proteoglycans (31-34), glycoproteins (35,36), and collagens (19,21,25,29) in the matrix of growth plate and artcular cartilages. The results have been consistent with many of the histochemical interpretations reviewed above, but their remarkable sensitivity has enabled better definition of the distribution and interactions of pericellular matrix components.

In the recent application of these methods, antibodies specific to degraded type II collagen have been used to map the in situ distribution of collagenolytic activity in degenerative human cartilages and bovine cartilage explant cultures treated with Interleukin-1 (IL-1) (37). A marked increase in pericellular degradation was observed in response to IL-1 implicating the chondrocyte as an active participant in the initial remodelling of the cellular microenvironment. Increased levels of type VI collagen have also been reported in osteoarthritic cartilage (24), while immunolocalisation studies have reported a more diffuse distribution of type VI collagen in degenerative cartilage (38) signalling important changes in the organisation and function of the chondron in the osteoarthritis.

Three matrix glycoproteins, Fibronectin (29,35,36), Thrombospondin (39), and Cartilage Matrix Glycoprotein (40) have also been immunolocalised in the pericellular microenvironment around articular cartilage chondrocytes. Elevated levels of fibronectin have also been reported in the matrix of osteoarthritic cartilage (41). Recent studies indicate that isolated articular (42) and rat chondrosarcoma (43) chondrocytes produce a hyaluronate rich "pericellular coat" around themselves In Vitro. Current evidence suggests that this hyaluronate coat is firmly attached to integral membrane receptors and may play a critical role in the assembly and sequestration of pericellular matrix components both In Vitro and In Vivo (42-44). The presence of membrane-matrix receptor sites, cell surface glycoproteins and several minor collagen and glycosaminoglycan species suggests that the formation of cell-matrix attachment sites must be important in stabilising the interaction between the chondrocyte and its pericellular microenvironment.

Agarose gel cultures have now been shown to maintain the chondrocyte phenotype in vitro and to promote the secretion and retention of a pericellular matrix which contains typical cartilage proteogycans and collagens (45,46). Aydelotte and colleagues have significantly expanded the potential use of this technique and have identified morphological and metabolic differences between sub-populations of chondrocytes extracted from the superficial, middle and deep layers of bovine articular cartilage (45,46). These techniques have now been refined to allow long term culture of agarose embedded chondrocytes which produce a distinct and easily identified pericellular matrix in vitro (47,48).

It is clear from the data briefly reviewed that each chondrocyte in adult articular cartilage is surrounded by a specialised matrix which contains a heterogenous mixture of proteoglycans, collagens and glycoproteins. Based on a variety of structural, metabolic and physical techniques it can be convincingly argued that the chondrocyte and its pericellular microenvironment truly represents the chondron and could conceivably act as the primary functional and metabolic unit of adult articular cartilage.

ARTICULAR CARTILAGE ULTRA STRUCTURE

• Detailed ultrastructural studies of the matrix organisation in adult articular cartilage have now been completed (49) and have recently been reviewed (50). In adult canine cartilage pericellular, territorial and inrerterritorial matrices were identified as was the distribution, potential origin, and function of articular cartilage matrix vesicles (49). When fixed under experimental compression the radial collagen fibres in the territorial matrix collapsed into a synchronised crimped waveform (14,50-52) while the pericellular matrix and capsule compact vertically and deform laterally in response to load. Data was also presented which suggests that matrix vesicles may be pumped out of the cellular microenvironment in response to dynamic loading (49). Further studies of testicular hyaluronidase treated cartilage showed a centripetal collapse of the pericellular capsule suggesting the normal anatomical position of the capsule could be maintained by the hydration of pericellular matrix proteoglycans (53).

Normal adult human cartilages have also been examined (54). Using a combination of scanning and transmission electron microscopy we demonstrated the presence of chondrons in the matrix of all the joint cartilages examined. Human chondrons had a similar orientation and organisation to those described in canine material, with the capsule showing a variety of fine fibrillar profiles, including filaments with a 100 nm period and thought to represent type VI collagen (54). We also demonstrated pores in the capsular weave which progressively descreased in size from the inner through to the outer margins of the capsule (54). Deformed matrix vesicles were often seen within the pores of the capsule,

throughout the territorial matrix, and releasing their contents in the interterritorial matrix (54).

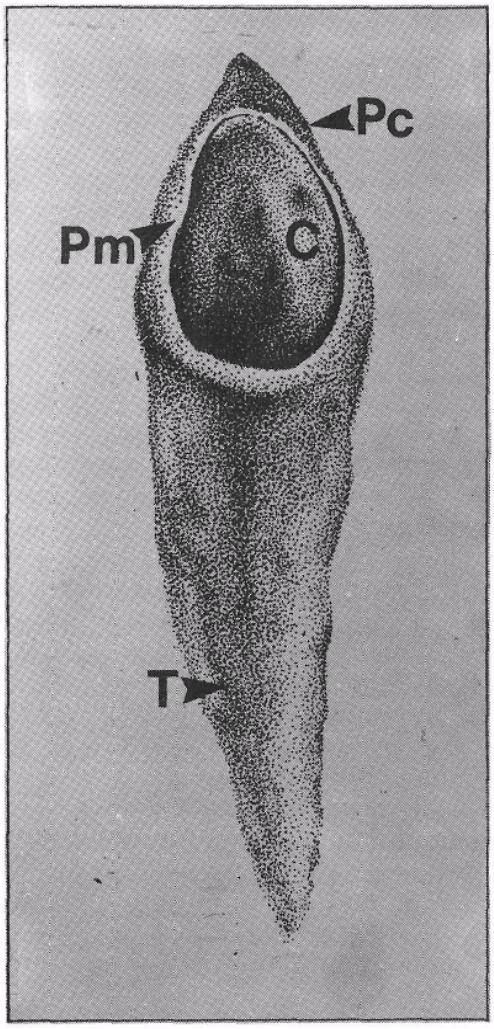
As a result of these physical and ultrasructural studies we hypothesized that functionally induced compression and deformation of the chondron could cause an adaptive "fluid" loss from pericellular matrix proteoglycans which could dampen the applied load and so provide hydraulic protection for the chondocyte. We also argued that the momentum of expressed fluid could generate the motive force (or microcirculation) of fluid necessary for the expulsion of cellulary derived matrix vesicles through the capsular pores and into the territorial and interterritorial matrices beyond the chondron (49,50,53-55). This hypothesis has gained widerspread support in recent years, but has yet to be substantiated experimentally.

DEVELOPMENT OF THE CHOHDROM EXTRACTION TECHNIQUE

• Szirmai was the first to suggest that chondrons could be extracted from hyaline cartilages by high speed homogenisation (2). In 1985 we expanded on Szirmai's observation and showed that fully differentiated chondrons could be released by serial low speed homogenisation from both fresh and fixed samples of adult canine and human tibial cartilages. Comparison with earlier structural data suggests the morphologies of the chondrons released are consistent with the capsular stmcture and its columnar organisation in the middle and deep layers of most vertebrate articular cartilages (49,50,53,54).

The details of chondron extraction methods have now been widely published (48,56-62). Briefly, a one gram sample of the diced cartilage was homogenised in 20ml of Phosphate Buffered Saline (PBS) at slow speeds (4000-6000 rpm) for short periods (1-10 min), diluted to 50ml with additional PBS, and the larger cartilage chips in the suspension allowed to settle. The flocculent supernatant was collected, the sediment washed again, the supernatans pooled and the process repeated until the sample was exhausted. Pooled supernatans were filtered through a descending series of nylon filters (1000-350 urn^2 and the filtered supernatant collected by centrifugation (500g, 10 min). Between 30 and 50% of the original cartilage sample was removed on filters > 350 u.m^2. The final pellet of filtered homogenate (< 350 m2) was resuspended in 10ml of fresh PBS and held at 4°C. Chondrons viewed as wet mounts or collected onto filters have now been assessed by phase contrast, differential interference contrast and scanning electron microscopy (56). Additional samples have been formed into small pellets, fixed and embedded in Epon and sectioned for transmission electron microscopy (48).Fig. 1.

The chondron homogenates produced by this method contain a heterogeneous mixture of intact viable chondrons, intact non-viable chondrons, rupted capsular ghosts lacking a chondrocyte, small cartilage chips (< 350 urn^2) and collagenous debris. Single chondrons (figure 1) consist of a chondrocyte and birefringent pericellular matrix enclosed within a moderately dense pericellular capsule which often extends from the "basal pole" to form a "tail" (56). The presence of these tails has only been established by the introduction of chondron extraction techniques and their function is completely unknown. However,the frequent discovery of frayed collagen bundles attached to the tail suggest it is strongly interdigitated with the radial collagen network which is itself firmly anchored in the calcified cartilage layer (50,51). In double (figure 2) and multiple chondrons (figure 3), each chondrocyte-pericellular matrix complex is separated from its adjacent neighbour by a dense capsular sheath continuous along the length of the linearly arranged chondron column. In these chondrons, the interconnecting segment between adjacent chondrocytes had a similar density and organisation to that of the tail and was often associated with frayed collagen bundles (56). These observations suggest that the dense parts of the pericellular capsule may be responsible for tethering the chondron and securing is position within



*FIGVRE /.*Single chondron as seen in phase contrast. The chondrocyte(C) is surrounded by a birefringent pericellular matrix (Prn) and is enclosed within a refractile pericellular capsule *(Pc).* This capsule is thin and densely compacted in the vicin-ity of the chondro-cyte-pericellular matrix complex but is thicker and more loosely organised in the tail (T) which frequently tapers from the basal pole of isolated chondrons

the matrix. Moreover, the continuity shown between these dense capsular areas and the thin compacted regions of the capsule which surround the cell would ensure the integrity of the chondrocyte-pericellular matrix interaction and maintain its relative position in the matrix during all phases of the loading cycle. Fig. 2 and 3.

Histochemical methods in conjunction with selective enzyme digestion and 4M guanidinium hydrochloride extraction have proved important for the routine assessment of the extracted chondron homogenate. For these studies we adapted cytological methods for collection of chondrons onto cellulose acetate filters. These filters could then be cleared during the mounting step and allowed detailed light microscopic examination on the distribution of proteoglycans and proteins in the ciiondron.

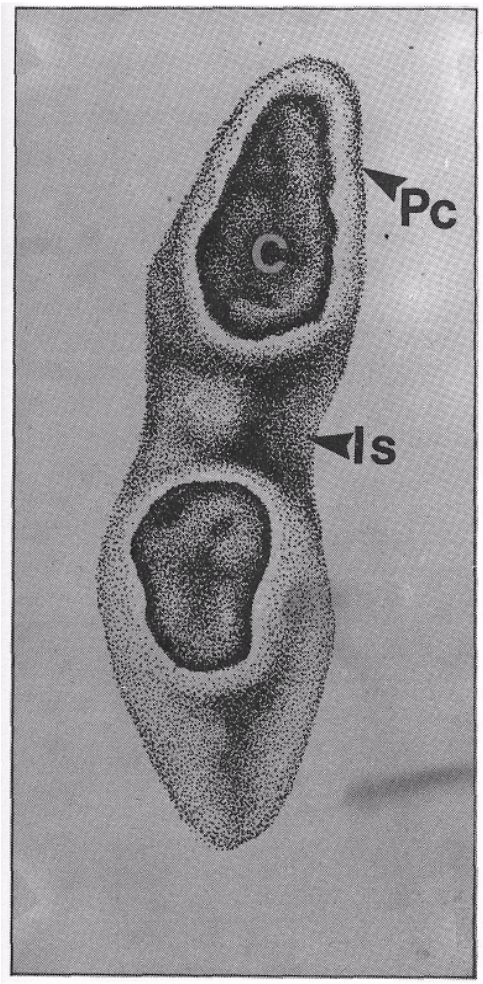
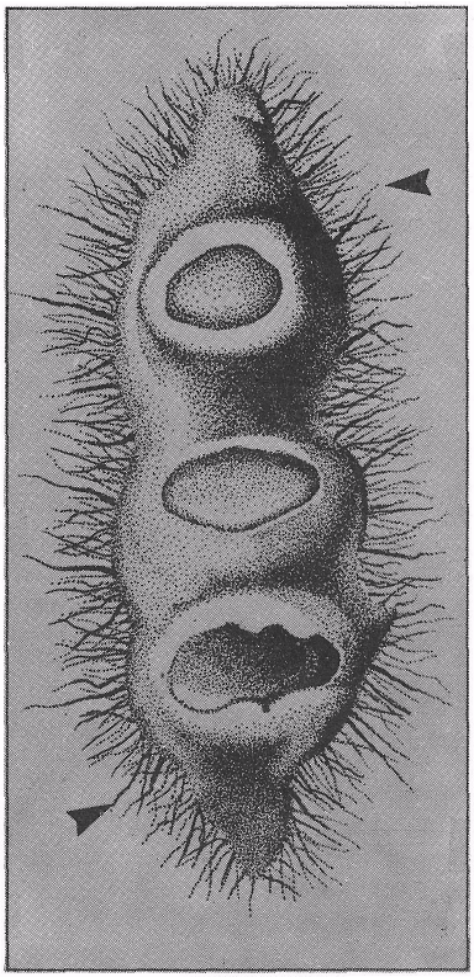


***FIGURE** 2.* Double chondron showing two chondrocytes (C) and their pericellular matrices separated from each other but held together as a unit by the continuity of the pericellular capsule (Pc). Again the capsule in the vicinity of the cell is thin and densely compacted while the interconnecting segment (Is) between adjacent chondrons is thicker and less densely organised.

FIGURES. Triple chondron showing thelinear organisation of chondrocytes typical of the deeper layers of adult articular cartilage. Each chondrocyte is retained in register by the continuity of the pericellular capsule. Frayed collagen bundles (arrowheads) were frequently associated with isolated linear chondron columns.



APPlICfiTiOHS OF THE ISOLATES CHOMDROH MODEL

- **Morphology.**

Initial studies have foccused on developing a variety of original techniques which have allowed us to use the isolated chondron as a model system to study the complexities of the cell-matrix interaction in a naturally occuring microanatomical unit of adult articular cartilage. Our first priority has been to identify the components which constitute a chondron, since an understanding of the composition, structure and organisation of normal chondrons is considered fundamental to the interpretation the changes which occur in the chondron during degenerative joint disease.

Using a battery of conventional connective tissue stains in conjunction with hyaluronidase, chondroitinase, keratanase and collagenase digestion, we have shown the chondron is rich in chondroitin sulphate, hyaluronan and protein, with lesser amounts of keratan sulphate and glycoprotein (57,58). These now routine methods are available to most research laboratories around the world and could be used for comparison, validation and quantitation of chondrons extracted from a variety of vertebrate articular cartilages.

Immunohistochemistry.

Immunohistochemistry has proved to be a critical technique in understanding chondron composition and organisation. In early experiments, isolated porcine and

rat chondrosarcoma chondrons were labelled in suspension with monoclonal antibodies to type IX and type II collagens. The results indicated that type IX is preferentially localised in the chondron and co-localises with type II collagen (59) which is consistent with previous interpretations of the nature of the type IX-type II collagen interaction.Further studies showed isolated canine chondons in suspension stain positively for type VI collagen. Intense staining was observed in the pericellular matrix immediately adjacent to the chondrocyte while a less intense, but quite distinct boundary staining was found at the outer margins of the capsule, in the tail, and in the interconnecting segments between columnated chondrons (60). Preliminary experiments using antibodies to a variety of matrix glycoproteins now suggest that fibronectin (58) is present in the chondron. Further studies with several different anti-laminin antibodies also suggest that laminin may form a component of the chondron, but these reproducible results have yet to be substantiated by biochemical analysis. Detailed examination of the distribution and role of fibronectin, laminin and other glycoproteins such as Cartilage Matrix Glycoprotein (40), thrombospondin (39), integrins, etc. in the chondron are planned in future experiments.

However, immunohistochemical studies on chondron suspensions requires repeated centrifugation during the numerous washing procedures necessitated by these techniques. In an effort to overcome these potentially damaging steps, we have been experimenting with a variety of techniques which allow us to immobilise isolated chondrons and better maintain the natural relationship between the chondrocyte and its pericellular microenvironment. These techniques have been adapted from a variety of disciplines and include colection onto filters, filter imprinting onto glass, and recently, the adaption of agarose gel culture methods to suspend chondrons in thin layers of low melting point agarose (61). ,>

Ultrastructure.

Studies of chondron ultrastructure initially proved difficult since random sectioning of centrifuged pellets was very time consuming. We have developed correlative light and electron microscopy techniques to study chondrons collected onto filters and stained en bloc with ruthenium red and ruthenium hexamine trichloride. Chondrons treated in this way are densely stained, and once embedded in resin, alow individual chondrons to be selected and marked with a diamond tipped objective. The inscribed chondrons were cut from the filter, carefuly trimmed and sectioned and examined by conventional and high voltage transmission electron microscopy. Data from these studies (48,58,61,62) show the preteoglycans in the pericellular microenvironment are attached to cell processes and the plasma membrane, and form an increasingly dense continuum which terminates abruptly at the outer margin of the pericellular capsule (48,58,62).

We have now adapted these techniques for immunoelectron microscopy using peroxidase-DAB-osmium and gold particle probes. The agarose gel technique described above has been particularly useful in this regard. Plugs of the chondron-agarose gels 5 mm diameter were cut from the culture dish and labelled with a wide variety of monoclonal antibodies to various protein and proteoglycan epitopes (61). Light microscopic examination of peroxidase-DAB labelled chondrons confirmed that antibody localisation was possible in this system, that chondron morphology was retained during agarose gel culture for up to 28 days, and that chondrons are rich in chondroitin-4-sulphate, chondroitin-6-sulphate, keratan sulphate and Hyaluronic Acid Binding Region epitopes (61).

Correlative light and electron microscopy coupled with immunolocalisation studies have therefore shown the chondron is complex microanatomical unit of adult cartilage and experiments using the isolated chondron as a structural model of the pericellular microenvironment are continuing.

Metabolism.

In vitro culture techniques are seen as pivotal to the future expansion of the chondron model and considerable effort has been directed at this problem. The successful introduction of agarose gel culture techniques into our repertoire of chondron handling methodologies has significantly expanded the potential use of the extracted material available and importantly, has lead to a significant reduction in the number of animals required for experimentation.

We have now introduced fluorescein diacetate as a chondrocyte viability marker (48,58), and have recently improved these methods to include a combination of 5,6-carboxyfluorescein diacetate and ethidium monozide which allow clear separation of live and dead chondrons. As a result, we have now begun to quantitatively assess the efficienty of our extraction procedures. However, homogenisation is a mechanically destructive process which, despite releasing the majority of chondrons in the preparation, results in yields of viable cells (chondrons) which are significantly lower than those achieved by traditional enzymatic extraction of the chondrocytes. Nevertheless, the use of 5,6-carboxyfluorescein diacetate as a viability marker viable shows yields of viable chondrons in the range of 0.24.0 X 10^6 per 1.0 gm of tissue. Further progress is required in the design of the cutting action of the homogeniser if we are to significantly improve the yield of viable cell for large scale studies of chondron metabolism.

Biochemical analysis of chondron components have

proved more difficult because of their small size and the presence of excessive amounts of other matrix materials. However, we have recently shown that the small fraction of proteoglycan (10-20%) not removed from cartilage by traditional extraction in 4M guanidinium hydrochloride could be histochemically localised exclusively in the chondron. Biochemical analysis of this resistant fraction revealed high levels of hyaluronate and chondroitin sulphate were retained in the chondron and suggests strong interaction, either chemical or physical, between the minor collagens and the proteoglycans which comprise the chondron (57).

Preliminary radiolabelling and autoradiographic studies have been completed (48,58,62) using chondron suspension labelled for periods of up to 24 hours with 35-S-sulphateor3-H-glycosamine.Autoradiographyonwhole mount preparations or sections of resin embedded chondrons revealed aggressive uptake of both labels and a discrete sequestration within the chondron (48,58,62). Obviously therefore, those chondrons which survive the homogenisation process intact and viable retain the appropriate phenotypic mechanisms necessary for the synthesis, sequestration and regulation of the pericellular microenvironment.

Osteoarthritis.

A critical component of our studies has been to examine the role and fate of the chondron during osteoarthritis since recent studies have shown this to be a key area in the initiation of degradative matrix changes (37). We have now examined chondrons extracted from spontaneous canine and human osteoarthritic knee joint cartilage (62). Comparative differences in chondron morphology were evaluated using differential interference and phase contrast microscopy, histological and histochemical techniques, and correlative light and electron microscopy. Chondrocyte viability was assessed using fluorescein diacetate and chondron metabolism investigated by autoradiography.

Comparison of representative histological sections with extracted chondron preparations indicates that the population of chondrons in the cartilage homogenate accurately reflects the diversity of chondron morphologies present in tissue sections (62). The most common morphological changes observed in osteoarthritic chondron preparations was the swelling and expansion of the cellular microenvironment, particularly in the "tail" and interconnecting segments between adjacent chondrons. Histochemical examination of osteoarthritic chondrons collected onto filters revealed concurrent changes in the fine collagenous weave of the pericellular capsule. Strong proteoglycan staining persisted within the swollen chondrons, but was consistently weaker and more patchy than comparably treated control preparations. This initial expansion of the chondron was followed by chondrocyte division and migration within the cellular microenvironment. Continued proliferation within the progressively expanding microenvironment results in the formation of a range of chondrocyte clusters which can be extracted intact and viable by homogenisation. Toluidine blue metachromasia progressively diminishes as these clusters increase in size, while ultrastructural studies show each chondrocyte within the cluster becomes surrounded by a dense calyx markedly different from the original chondron. Pericellular collagenolysis has now been identified as a key factor in the initiation of osteoarthritic changes (37). Our studies have identified three potential steps in the process of chondron remodelling. Firstly, we suggest that enzymatic disruption of the fine collagenous weave of the pericellular capsule remodels the physically restrictive microenvironment around the chondrocyte allowing increased hydration of pericellular proteoglycans causing the chondron to swell. Secondly, we argue that morphological and physicochemical changes in the organisation of the pericellular microenvironment then promotes division of the progenitor chondrocyte within the chondron. Finally, mitotic proliferation continues in parallel with increased proteoglycan synthesis and expansion of the pericellular microenvironment to form the large chondrocyte clusters typical of osteoarthritic pathology. While the mechanisms responsible for the initiation and progression of these changes have yet to be identified, the isolated chondron model offers the potential to study the role of the pericellular micrienvironment in the etiopathogenesis of degenerative joint disease.

Conclusion.

Articular cartilage is a tissue of critical importance for the proper health of articulating joints since it functions to transmit and redistribute the extreme loads generated during normal daily activity. Two major diseases are associated with the failure of cartilage function. Rheumatiod arthritis is a systematic disease normally affecting 1-3% of the population, and although is etiology is unknown, it is characterised by synovial inflammation and severe erosion and/or remodelling of the cartilage and bone in the joint. Conversely, osteoarthritis is often rather simplisticly refered to as a "wear and tear" disease which affects large numbers of individuals in the community, particularly from middle age and beyond. A multiplicity of etiological factors have been implicated in the onset of osteoarthritis but despite significant advances in cartilage research over tha last three decades we do not yet a clear indication as to the mechanism operating in the etiopathogenesis of this disease.

What is clear however, is that once skeletal maturity has been achieved, the chondrocyte is solely responsible for the maintenance and repair of the extracellular matrix

which surrounds it. This matrix represents more than 90% of the total tissue volume and is actually responsible for the transmission of the applied load to the underlying bone.Ultimately therefore, the health of the cartilage matrix must depend on the health of the small number of chondrocytes which produces it. Cartilage in fact has one of the lowest cell densities per unit volume of any tissue and it follows that functional, developmental or genetic abnormalities which might compromise chondrocyte viability or metabolism will similarly compromise matrix integrity and ultimately the mechanical function of the joint.

Over the last ten years we have identified and defined a specialised microenvironment arou nd each chond rocyte in adult articular cartilage and presented convincing evidence to show that this microanatomical structure within cartilage could represent the chondron originally described by Benninghoff in 1925. We subsequently discovered and developed a technique to extract and isolate chondrons from low speed homogenates of adult articular cartilage and have shown that they are sufficiently mechanically robust to enable extraction of chondrons containing viable chondrocytes. We have now introduced several original techniques to our studies of the isolated chondron and have used these to significantly advance our understanding of the structure, composition and function of this important physiological unit of cartilage. More importantly perhaps, we have recently discovered that significant remodelling of the pericellular microenvironment occurs in chondrons extracted from human and canine osteoarthritic cartilage and provides the basis for future studies on the role of chondrocyte - pericellular matrix in degenerative joint disease.

It is clear from the data briefly reviewed that each chondrocyte in adult articular cartilage is surrounded by a specialised matrix containing a heterogenous mixture of proteoglycans, collagens and glycoproteins. Based on a variety of structural, metabolic and physical techniques it can be convincingly argued that the chondrocyte and its pericellular microenvironment represents the chondron and could conceivably act as the primary functional and metabolic unit of adult articular cartilage. The concept of the chondron has truly come of age.

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Address for correspondence:
C. Anthony Poole
Department of Anatomy
University of Auckland Medical School
Private Bag,
Auckland,
New Zealand.