CHAPTER II
LITERATURE REVIEW

Structure of Diarthrodial Joint

The equine joint structure consists of the articulating surfaces of large bones covered by articular cartilage, the synovial membrane, the fibrous joint capsule, a cavity containing synovial fluid, and associated ligaments (figure 1 and 2). It has two major functions: (1) to enable movement and (2) to transfer load. The structure of the synovial joint is designed to facilitate these two major functions (Todhunter, 1996). The joint capsule composes of outer fibrous layer, which is continuos with the periosteum or perichondrium, and inner synovial membrane, which lines the synovial cavity where articular cartilage is not present. The synovial membrane is essentially highly vascularlized modified connective tissue and, such as, manifests a typical inflammatory response (McIlwraith, 1982). The nutrients required by the joint tissues are supplied by these blood vessels (Corporate Country Bayer Group Community, 2001). The capsule has sensory nerve ending and, in most cases, the pain associated with joint inflammation arises from the joint capsule . Strong joint ligaments surround and connect the bone ends, providing stability and protection for the joint. The ligaments also contribute to keep flexion and extension of the joint within certain limits. The joint cavity is filled completely by synovial fluid, which acts as a means of transportation of the nutritive substances required by the cartilage and lubricates the soft tissues of the joint to prevent inflammation and pain (Viitanen, *et al.,* 2000). Articular cartilage, which provides for frictionless sliding and the absorption/distribution of forces on the joint, covers the end of bones. It depends on circulating joint (synovial) fluid to stay healthy. The synovial membrane surrounds the joint and functions to produce synovial fluid .

The articular cartilage consists of hyaline cartilage, the matrix, which is a complex of collagenous fibrils, and a highly hydrated ground substance containing proteoglycans,

glycosaminoglycan, and glycoproteins. The translucent, glasslike (hyaline) appearance of articular cartilage is due primarily to its high water content (70% by weight in mature cartilage, and approaching 80% in neonatal cartilage) and the very fine structure of its collagen fibril network. On a dry weight basis, articular cartilage contains about 50% collagen, 35% proteoglycan, 10% glycoprotein, and other minor components (Todhunter, 1996).

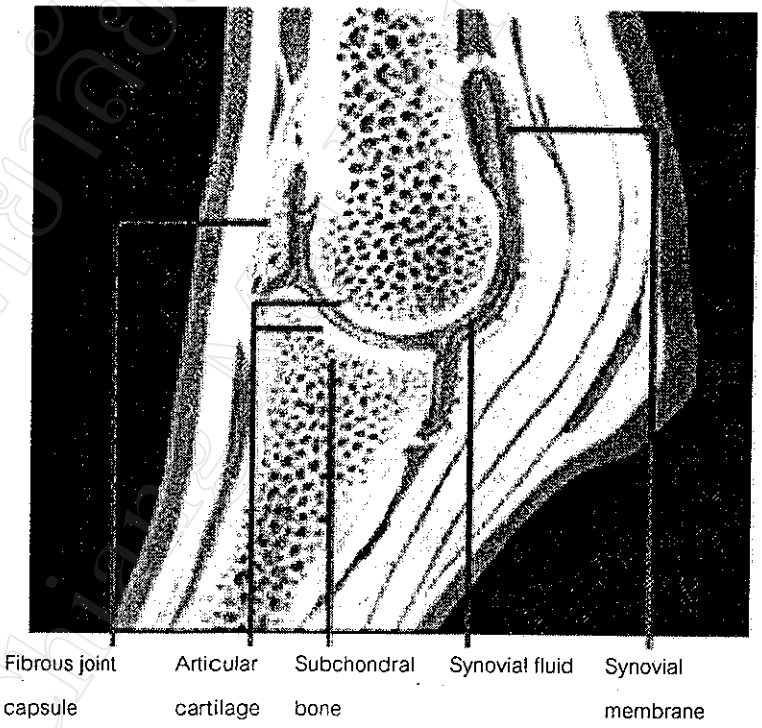


Figure 1 Structure of diarthrodial joint (Corporate Country Bayer Group Community, 2001 [Online]. Available:http://www.yourhorseshealth.com/joint_therapy/struct. .html)

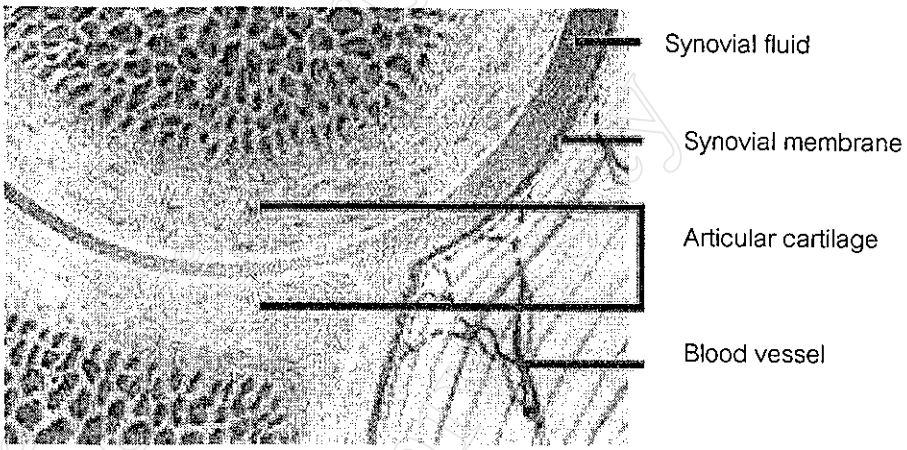


Figure 2 Structure of diarthrodial joint (close up) (Corporate Country Bayer Group Community, 2001[Online]. Available: http://www.yourhorseshealth.com/joint_therapy/struct.html)

Articular cartilage

Hyaline cartilage covering the articular surfaces of diarthrodial joints is a highly specialized connective tissue with a biomechanical function that is particularly suited to bearing compressive load (Todhunter, 1996). It is a highly specialized tissue that in higher vertebrates forms the template of long bones during development and is retained in the adult in selected sites, particularly on the weight-bearing surfaces of articular joints. Cartilage is a tissue in which the cells (chondrocytes) comprise only a few percent of the volume, and the major part of the tissue is a highly organized and expanded extracellular matrix. The important biomechanical properties of this tissue are the result of the composite structure of the extracellular matrix, which consists of a dense network of fine collagen fibrils, which are responsible for the form and tensile properties of the tissue, and a high concentration of aggregated proteoglycan (predominantly aggrecan), which binds with hyaluronan and draws water into the tissue by osmosis and exerts a swelling pressure on the collagen network (Hardingham and Fosang, 1992). It is the retention of aggrecan in complex form with hyaluronan within the inextensible collagen network that causes the swelling pressure and makes the tissue ideal for resisting compressive load with minimal deformation, thereby supporting its function as a tough and resilient load-bearing surface (figure 3) (Hardingham, 2002). Collagens form a dense fibrillar network that is embedded with a very high concentration of proteoglycan aggregated with hyaluronan. Proteoglycan, as a result of their polyanionic glycosamonoglycan chains, creates a large osmotic swelling pressure due to the Gibbs-Donnan equilibrium, which draws water into the tissue and expanded the collagen network. The major constituents of cartilage matrix, namely collagen fibrils, the aggregating proteoglycans, and hyaluronan have very different structures and rates of turnover, reflecting their different function in the joint (Todhunter, 1996).

Most of the collagen in articular cartilage is type II (85 to 90% of the total), and small amounts of types VI, IX, XI, XII, and XIV are also present. Type II collagen is generally thought to provide the tensile strength of the articular cartilage. Equine type II

collagen has a structure similar to that of other species and has a higher hydroxylation of lysine residues and more glycosylation than equine type I collagen (Todhunter, 1996).

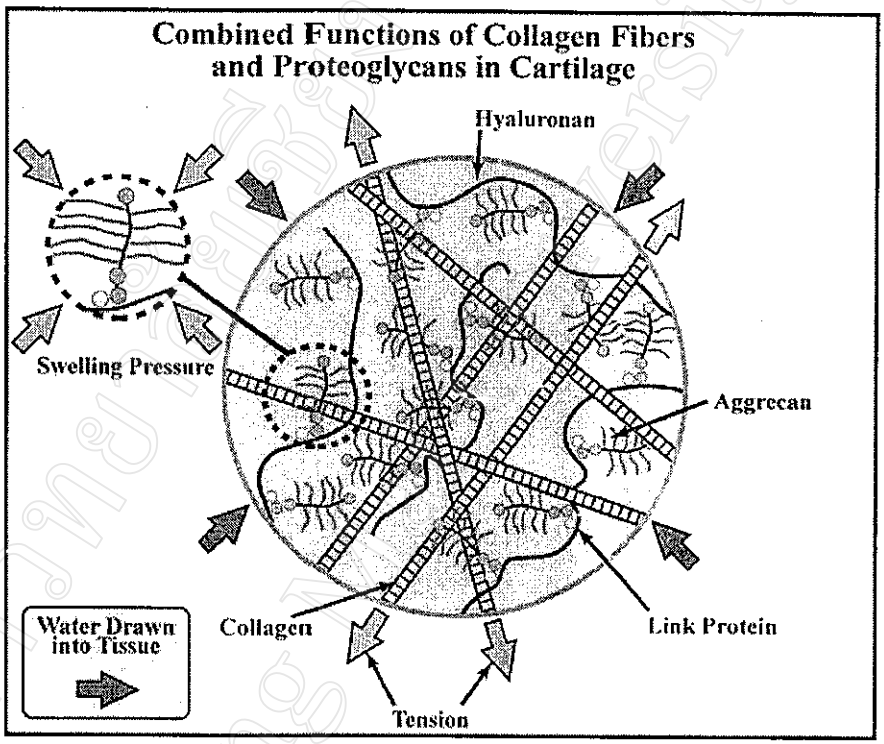


Figure 3 Organization of extracellular matrix of articular cartilage (Hardingham, 2002 [Online]. Available: http://www.glycoforum.gr.jp/science/hyaluronan/HA05E.html)

It was also found that articular cartilage is hetrogenous with variation in microstructure between different joints, between weight-bearing and non-weight-bearing areas, and between young and adult animals (Todhunter, 1996). Under microscopic study, articular cartilage is subdevided into three unmineralized zones (I to III), which are delineated from the calcified cartilage (zone IV) by the tidemark. The lower boundary of the calcified cartilage is the cement line formed during endochondral ossification of the articular epiphyseal growth plate at maturity. In adult animal, zone I (superficial or tangential zone) has the highest cell density. The chondrocytes are relatively small and flat, oriented with the long axis parallel to the surface. Zone II (transitional zone) shows larger and rounded cellular profiles. In zone III (radiate zone), the cells are larger and arranged with their long axes perpendicular to the surface (Todhunter, 1996).

It has been suggested that the zonal variations in cartilage represent a functional adaptation to the mechanical requirement of the different layers. This is overly simplistic, but the superficial layer does appear to form a wear resistant protective diaphragm that can withstand tension in the plane of articular surface. In contrast, the fibrils in the middle and deep zones are organized to provide increased resilience to compressive loading because of their tendency to perpendicular alignment to the tidemark. The concentration of proteoglycan increases with increasing depth from the articular surface, and therefore the collagen fibrils are more concentrated at the surface, where they are required to resist tensile strain during weight bearing. The increased concentration of proteoglycan in the deeper cartilage is probably required to resist compression (Todhunter, 1996). During growth, the epiphyseal and growth plate cartilage undergo interstitial growth and endochondral ossification. During these processes, the extracellular matrix of the cartilage is degraded and the primary spongiosa is formed. This degradation process results in releasing of cartilage-containing products into the blood. Presumably, degradation of epiphyseal and growth plate cartilages would be highest when the rate of longitudinal bone growth is highest, and the proteoglycan fragment was found to peak approximately 10 weeks of age (Todhunter, *et al.*, 1997).

Chondrocytes synthesize, organize, and regulate the composition of a complex pericellular, territorial, and interterritorial matrix. At each stage of growth, development, and maturation, the relative rates of matrix synthesis and degradation are adjusted to achieve net growth, remodeling, or equilibrium (Todhunter, 1996). Any failure to maintain the proteoglycan content of articular cartilage will adversely effect its biomechanical properties, which leading to degeneration of articular cartilage (Hardingham, *et al.*, 1994). Many authors have described the variations in the composition of cartilage within individual joints. In order to avoid the complications of sampling variation between specimen used in cartilage component studies (Fuller, *et al.*, 1996).

Proteoglycan and glycosaminoglycan

Other than collagen, proteoglycan is the major solid components of articular cartilage. It has important role to act as a sponge that absorb the water into cartilage matrix and support the collagen network, this allow cartilage resist compressive load without damage. Proteoglycan is a class of glycosylated proteins, which have covalently linked sulfated glycosaminoglycan, (i.e., chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate). The protein component of proteoglycans is a core protein, which directs the biosynthesis of proteoglycans to different molecular construction and functions (Yanagishita, 2002). Biological functions of proteoglycans derive primarily from those of the glycosaminoglycan and protein component of the molecule. Glycosaminoglycans assume extended structures in aqueous solutions because of their strong hydrophillic nature based on their extensive sulfation, which is further exaggerated when they are covalently linked to core proteins. They hold a large number of water molecules in their molecular domain and occupy enormous hydrodynamic space in solution (Yanagishita, 2002). Many researcher have tried to identified proteoglycan subpopulation either directly from cartilage and indirectly from body fluids by using immunolocalization or separating component of proteoglycan in order to study the cartilage proteoglycan metabolism (McIlwraith, 1982; Fuller,*et al.*, 1996; Platt, *et al.*, 1998). Proteoglycan, consists of one or more glycosaminoglycan chains,

which are sulfated polysaccharides made of repeating disaccharides (typically a repeat of 40-100 times) (Yanagishita, 2002), covalently attached to a protein core. Glycosaminoglycan components in proteoglycan are shown in table 1.

Table 1 Glysosaminoglycan components of proteoglycan found in cartilage (adapted from Todhunter, 1996)

Glycosaminoglycan name	Primary core protein	Wet weight of cartilage (%)*	Wet weight (nmol/gm)*	Other names	Repeating unit in glycosaminoglycan
Chondroitin sulfate	Aggrecan	5-10	1-10	PG-LA1	*N*-acetylgalactosamine-β(1-4)-glucuronic acid-β(1-3)
Dermatan sulfate	Decorin	0.03-0.12	0.3-0.6	PG-S2 PGII	*N*-acetylgalactosamine-β(1-4)-glucuronic acid-β(1-3)
	Biglycan	0.06-0.24	0.25-0.5	PG-S1 PGI	*N*-acetylgalactosamine-α(1-4)-iduronic acid-α(1-3)
Keratan sulfate	Aggrecan			PG-LA1	*N*-acetylglucosamine-β(1-3)-galactose-β(1-4)
	Fibromodulin	0.1-0.3	1.5-5	59-kDa protein	*N*-acetylglucosamine-β(1-3)-galactose-β(1-4)
Hyaluronan	None	0.05-0.25	0.03-0.08	Hyaluronic acid	*N*-acetylglucosamine-β(1-4)-glucuronic acid-β(1-3)

* Figure for nmol/g are approximate; these molecules are particularly heterogenous, and estimates of their molecular mass vary by as much as 50%. In addition, calculation of the percentage wet weight of cartilage are prone to error based on the efficiency of extraction. To further complicate matters, the amounts of most components vary with age and exact tissue source. These figures are thus only rough guide.

Aggrecan

The large aggregating proteoglycan, which is found the most abundant in cartilage, is called "Aggrecan" (Hardingham and Fosang, 1992). Aggrecan provides a strongly hydrated space filling gel that contributes to the physical strength of

cartilagenous tissue (Yada, 2002). The aggrecan structure consists of an extended protein core, which many chondroitin sulfate and keratan sulfate chains are attached. This forms a densely substituted branched or "bottle brush" structure (figure 4), which provides a highly focused concentration of polyanion, which is fully hydrated and spacefilling (Hardingham, *et al.*, 1994). It contains several features, which help to provide the specialised biomechanical properties of cartilage (Hardingham and Fosang, 1992).

Aggrecan, whose molecular mass is about 2500 kDa. Its distribution pattern is relatively restricted to several tissues including cartilage, and also brain, aorta and tendon. The core protein of 210-250 kDa binds with hyaluronan and forms supramolecular complex together with link protein (Yada, 2002).

A core protein has high molecular weight (approximately 250 kDa) encoded by a single gene that is expressed predominantly in cartilaginous tissue (Hardingham and Fosang, 1992). It has 3 globular and 2 extended domains (figure 4). And as described above, it is highly glycosylated with approximately 90% carbohydrate, mainly in 2 types of glycosaminoglycan chains, the major chondroitin sulfate and minor keratan sulfate, which are typically approximately 20 kDa each. The chondroitin sulfate chain, either 4-sulfated, 6-sulfated, or usually both, are all attached to the long, extended domain between globular domain 2 and 3, but the keratan sulfate chains are more widely distributed. They are most abundant in a keratan sulfate-rich region just C-terminal to the G2 domain (Hardingham, 2002).

The globular N-terminal G1 domain, of aggrecan contains an immunoglobulin-like binding site (Heinegard and Hascall, 1974; Hardingham, 1981), which has a high affinity for hyaluronan when link protein appears and is responsible for the formation of aggregates. As hyaluronan is a long, unbranched chain with molecular weights of up to several million, each chain can bind a large number of aggrecans to form aggregates up to several hundred million in molecular weight (Hardingham, 2002).

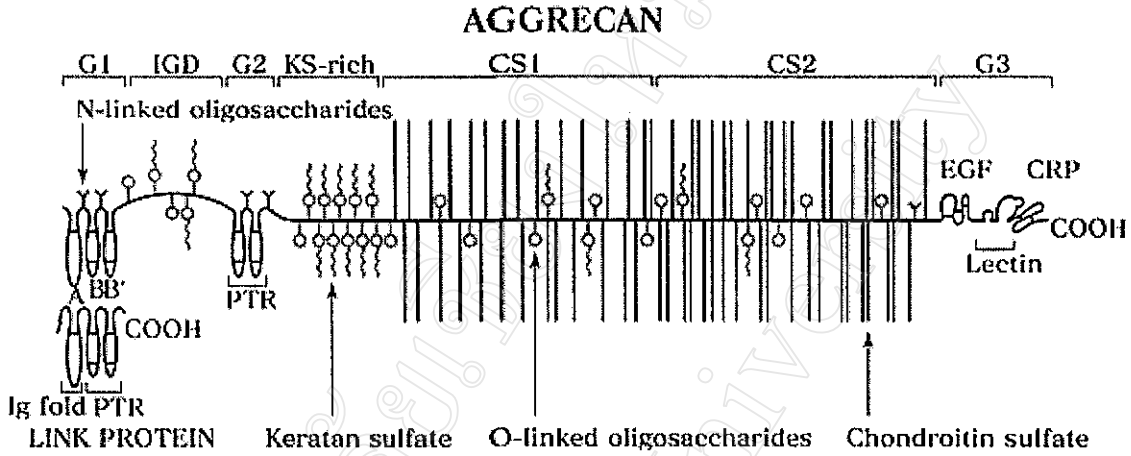


Figure 4 Cartilage proteoglycan (aggrecan) structure (Hardingham, 2002 [Online]. Available: http://www.glycoforum.gr.jp/science/hyaluronan/HA05E.html): G1 = globular domain 1, G2 = globular domain 2, G3 = globular domain 3, IGD = interglobular region, CS1 = chondroitin sulfate region 1, CS2 = chondroitin sulfate region 2, EGF = epidermal growth factor-like sequences, CRP = complement regulartory protein-like module, PTR = proteoglycan tandem repeat

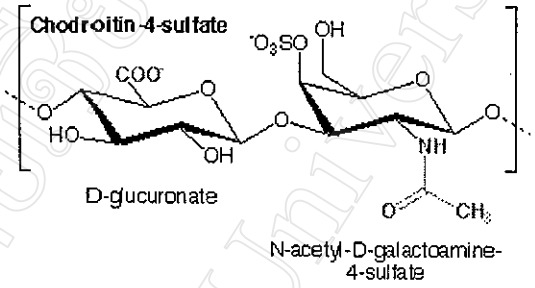
Bayliss and his coworkers also found that the rate of incorporation of aggrecan into aggregates is much slower in mature cartilage than in tissue obtained from younger individuals (Bayliss, *et al.*, 2000). Aggrecan is synthesized and secreted continuously by chondrocytes, and it follows the same intracellular pathways of synthesis as other secretory proteins. The mRNA is translated on membrane-bound ribosomes into the rough endoplasmic reticulum, followed by translocation to the Golgi for the main steps of O-glycosylation and glycosaminoglycan chain synthesis. There is no intracellular storage of the finished molecule prior its release. Link protein, although it is less glycosylated and has no glycosaminoglycan chains, is also synthesized along the same intracellular pathway (Hardingham, 2002). Aggrecan and link proteins thus encounter hyaluronan only after their secretion by the chondrocytes into the extracellular matrix (Hardingham, *et al.*, 1994). Therefore, aggregation is an extracellular mechanism for the assembly of aggrecan into higher order structures. As this favors retention of aggrecan within the cartilage extracellular matrix, this process plays a major role in maintaining the large concentration of aggrecan in the matrix required supporting the tissue's biomechanical function (Hardingham, 2002).

Chondroitin sulfate

The aggrecan structure consists of an extended protein core to which many chondroitin sulfate and keratan sulfate chains are attached. This forms a densely substituted branched or "bottle brush" structure (figure 4). The major glycosaminoglycan is chondroitin sulfate, which is formed of repeating disaccharide subunits of glucuronic acid (glcUA) and N-acetylgalactosamine (galNAc) (Yanagishita, 2002; Brown, *et al.*, 1998) (figure 5). Sulfation of glycosaminoglycans in chondroitin sulfate chains is usually regular, one sulfate per disaccharide throughout the chain (Yanagishita, 2002). The galNAc portion may be unsulfated or sulfated at the 4 or 6 position (ΔdiC-6-S or ΔdiC-4-S) (Brown, *et al.*, 1998). There were significant age-related changes in sulfating pattern of chondroitin sulfate chains (Brown, West *et al.* 1998). The ΔdiC-6-S:ΔdiC-4-S molar ratio of both the endogenous and newly synthesized chondroitin sulfate suggests a slight but

significant downward trend with increasing age of the specimen. Moreover, at each age the disaccharide ratio of newly synthesized chondroitin sulfate chain is generally slightly lower than that of the endogenous chain (Platt *et al.*, 1998)

A



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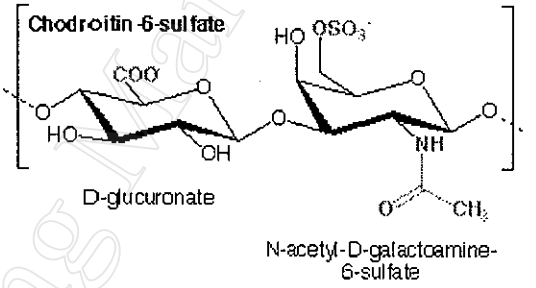

Figure 5 The repeating disaccharide subunit of chondroitin sulfate (A= chondroitin-4-sulfate, B= chondroitin-6-sulfate) (2002 [Online]. Available: http://208.7.154.206/gmoyna/biochem341/glucosaminoglycans.gif)

Keratan sulfate

Keratan sulfate, another glycaosaminoglycan found in aggrecan, consists of the repeating disaccharide of N-acetylgalactosamine-β(1,3)-galactose-β(1,4). Keratan sulfate concentration in body fluids was indicative of articular cartilage catabolism in response to joint insult (Todhunter, *et al.*, 1997). Because of this, there were many researchers that attempt to apply keratan sulfate as a marker of joint disease (Hardingham, 1995; Sharif, *et al.*, 1995; Todhunter, *et al.*, 1997; Innes, *et al.*, 1998; Okumura and Fuginaga, 1998; Innes, *et al.*, 1999). Keratan sulfate was also reported in concentration associated with age. A study in foal, serum keratan sulfate concentration was high from 1 week after birth to 3 months of age indicated that cartilage catabolic activity is higher in developing foals up to 3 months of age. This suggests that the catabolic activity of cartilage, the rate of catabolism, and possibly anabolism are high in the initial growth stage in foals (Okumura, *et al.*, 1997).

Hyaluronan

Much of the hyaluronan is associated with aggrecan, in the form of large aggregates of as many as 100 aggrecan monomers associated with a single hyaluronan molecule. It is the only glycosaminoglycan that is not sulfated (Todhunter, 1996). Hyaluronan is also present in all vertebrates, perhaps arising in animals with notochords. Hyaluronan is a major constituent of the extracellular matrices in which most tissues differentiate. It is also an essential component of many extracellular matrices in mature tissues. In some cases, hyaluronan is a major constituent; as, for example, in the vitreous of the human eye, or in the matrix produced by the cumulous cells around the oocyte prior to ovulation, or in synovial joint fluid and in articular cartilage. Hyaluronan is present at approximate 1 mg/g wet weight in hyaline cartilages, enough to fill the tissue volume in the absence of other constituents. Hyaluronan is unique among the proteoglycans in that it is not synthesized attached to a core protein. It has been shown to impart many other physiologic and pathologic mechanisms, by modulating chemotactic, proliferative, and phagocytic response of various inflammatory cells; regulating oxidative damage; and inhibiting release of proteoglycans from cartilage.

Chemical structure of hyaluronan composed of the uronic acid and aminosugar in the disaccharide, which are D-glucuronic acid and D-N-acetylglucosamine, and that they are linked together through alternating beta-1,4 and beta-1,3 glycosidic bonds (figure 6). The number of repeat disaccharides, n, in a completed hyaluronan molecule can reach 10000 or more, a molecular mass of approximately 4 million Dalton (Hascall and Laurent, 2002). Each chain of hyaluronan can bind a large number of aggrecans to form aggregates up to several hundred million in molecular weight. The binding of each aggrecan to hyaluronan is further stabilized by a small glycoprotein (45 kDa) referred to as link protein (Hardingham, 2002)

Chondrocytes actively synthesize and catabolize hyaluronan throughout the lifetime of the tissue. Hyaluronan is not synthesized within these same compartments within the cell, but is formed by a synthase enzyme that appears to be located in the plasma membrane, such that the elongating hyaluronan molecule is secreted directly into the extracellular matrix (Hardingham, 2002). Synthesis is usually balanced by catabolism, thereby maintaining a constant concentration in the tissue. Metabolic studies have shown that the half-life of a hyaluronan molecule in cartilage is normally 2-3 weeks (Hascall and Laurent, 2002).

The concentration of hyaluronan in tissue (Thonar, *et al.*, 1978), synovial fluid (Hilbert, *et al.*, 1984; Tulamo, *et al.*, 1994; Tulamo, *et al.*, 1996), and serum (Sharif, *et al.*, 1995; Laurent, *et al.*, 1996) was measured in order of studying cartilage metabolism, especially in joint diseases such as osteoarthritis. A simple assay for determining hyaluronan in human serum was developed using biotinylated hyaluronan-binding protein (s) (Surankul, 1998). In addition, hyaluronan is carried from the joint to the blood by lymph, and taken up rapidly by the liver, although a minor amount may be removed by kidney. Thus hyaluronan level may also be a marker of liver disease (Laurent, *et al.*, 1996).

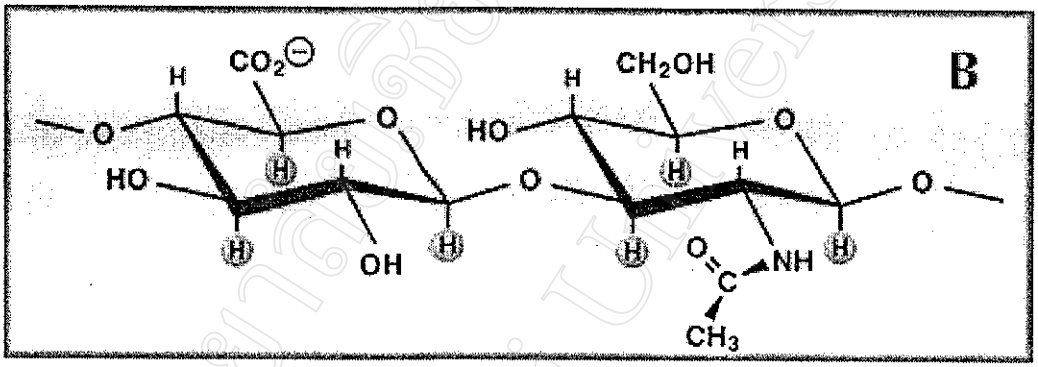

Figure 6 The repeating disaccharide subunit of hyaluronan (Hascall and Laurent, 2002 [Online]. Available: http://www.glycoforum.gr.jp/science/hyaluronan/HA01E.html)

Monoclonal antibodies against proteoglycan fragments

Several assays have been developed to recognize proteoglycan fragment from aggrecan (Garnero, *et al.*, 2000) in order to study the catabolism or anabolism of cartilage. Glycosaminoglycans, such as chondroitin sulfate and keratan sulfate, have been most frequently chosen in case of proteoglycan fragment determination.

Biochemical studies to determine the quantity of sulfated glycosaminoglycans in the synovial fluid have been completed; however, these methods have the disadvantage of identifying all sulfated glycoaminoglycans in the fluid despite their origin. Monoclonal and polyclonal antibodies targeted to epitopes specifically located on cartilage proteoglycan and collagen fragment present in the synovial fluid and serum have given researchers a more specific and sensitive tool for studying articular cartilage metabolism and pathology (Ray, *et al.*, 1996). Concepts have been made to estimate articular cartilage breakdown in the joints by measurement of keratan sulfate and chondroitin sulfate by immunological means.

Over the past decade, researchers have attempted to specifically identify and quantitate types and amounts of articular cartilage components that are liberated into synovial fluid and ultimately into the serum as articular cartilage degeneration occurs. Immunologic methods appear to provide the most sensitive means to achieve this goal. Polyclonal and, most recently, monoclonal antibodies have been produced against various parts of aggrecan and other molecules that are released from the cartilage. By definition, monoclonal antibodies are produced by a single clone of B-lymphocytes and are therefore of a single specificity (Ray, *et al.*, 1996). Once a monoclonal or polyclonal antibody to a specific epitope, an area on the surface of an antigenic molecule against which an immune response is directed (Ray, *et al.*, 1996), has been produced, the amount of epitope can be measured using a radioimmunoassay or ELISA (Ray, *et al.*, 1996). Epitopes have been identified in a number of areas of the proteoglycan monomer including chondroitin sulfate and keratan sulfate, the hyaluronan binding region (G1), the chondroitin sulfate-attachment region, the keratan sulfate region, the G3 globular domain

and link protein (Ray, *et al.*, 1996). Monoclonal antibodies against each epitope of cartilage proteoglycan were presented in table 2.

Focused on chondroitin sulfate epitopes, monoclonal antibody 3B3 recognizes its chondroitin sulfate epitope, a non-reducing terminal unsaturated uronic acid residue adjacent to N-acetylgalactosamine-6-sulfate after the chondroitin sulfate chain has been digest with chondroitinase. This epitope is denoted as 3B3(+) (figure 7) since one requires that chondroitin sulfate proteoglycans be predigested with chondroitinase to generate its specific epitope. Chondroitinase ABC increased the detectable epitope by 20-100 fold in serum samples analyzed in a previous study (Pothacharoen, 2000). However, this antibody also recognizes a natural mimotope, a biochemical structure that mimics the epitope recognized by a given antibody, containing a saturated glucuronic acid residue at the non-reducing terminal adjacent to N-acetylgalactosamine-6-sulfate, that occur in chondroitin sulfate chains of proteoglycan isolated from osteoarthritic cartilage (Slater, *et al.*, 1995) (Caterson, *et al.*, 1995). When antibody 3B3 is use without chondroitinase pretreatment immunoreactivity with the native mimotope structure is designated 3B3(-) (figure 7) (Caterson, *et al.*, 1995).

Horses with induced arthritis showed a strong 3B3(-) epitope immunolocalization of their cartilage when compare with control and placebo groups (Todhunter, *et al.*, 1996). A study analyzed 3B3(-) epitope in proteoglycan extracted from cartilage of human patient with many arthritic diseases including osteoarthritis showed a similar result as in horses (Slater, *et al.*, 1995). Researches studying in synovial fluid also showed an increased 3B3 in human osteoarthritic knee (Belcher, *et al.*, 1997) and in human traumatic joint (Hazell, *et al.*, 1995). In serum, 3B3(+) was significantly higher level in osteoarthritic and rheumatoid human patients than in normal (Pothacharoen, 2000). Therefore, 3B3 epitope was proved that it might be used for detecting the alteration of cartilage metabolism in joint diseases.

Monoclonal antibody 846, specific to chondroitin sulfate epitope (846) has been described. The epitope found only the largest, fully aggregatable aggrecan molecules. That it is present on newly synthesized molecules is indicated by a recent study (Frisbrie,

et al., 1999). Serum and synovial fluid concentrations of epitope 846 were found to be associated with osteochondrosis. Increase in concentration of epitope 846 suggests that increased synthesis of cartilage aggrecan (Frisbrie, *et al.*, 1999).

Monoclonal antibody WF6 recognizes an epitope in a native chondroitin-6 sulfate and competitive ELISA was developed to detect the WF6 epitopes in human serum using aggrecan (A1D1 fraction) (Pothacharoen, 2000). Tiengburanatam described preparation of the WF6 monoclonal antibody in a previous study (Tiengburanatam, 1996). Pothachareon (2000) studied this epitope and found that it was higher in osteoarthritic patients than in normal serum, and it was also significantly higher in rheumatic arthritis serum (Pothacharoen, 2000). WF6 epitope was discussed that it might be able to reflect the degradation of cartilage without digesting with chondroitinase.

Monoclonal antibodies against keratan sulfate such as 1/20/5D4 (Okumura, *et al.*, 1997; Innes, *et al.*, 1998; Innes, *et al.*, 1999) and 1/14/6H9 (Okumura and Fuginaga, 1998) have also been studied and described. It was found that horses with osteochondral chip fracture, other closed intraarticular fracture, inflammatory arthritis (synovitis), infectious arthritis, or osteochondrosis had significantly higher plasma keratan sulfate concentration than did clinically normal horses, but horses with osteoarthritic did not. It is possible that cartilage from these joints may have been in an advanced stage of disease characterized by loss of cartilage (Todhunter, *et al.*, 1997). However, Okumura (1998) found that serum keratan sulfate in osteoarthritis was significantly higher than that in normal horses, while no significant difference was found in keratan sulfate levels of synovial fluid between normal and osteoarthritic horses (Okumura, et *al.*, 1998).

Table 2 Monoclonal antibodies directed against the polysaccharide attachment region of cartilage proteoglycan (adapted from ; Seikagaku corporation, 1999)

Clone	Immunogen	Specificity	Antibody subclass
Chondroitin sulfate family			
CS-56	Ventral membrane of gizzard fibroblast	Chondroitin sulfate	IgM
MO-225	Proteoglycan from chick embryo limb bud	Chondroitin sulfate type D	IgM
MC21C	Adult rat bone protein	Chondroitin-6-sulfate	IgM
LY111	Chicken type IX collagen containing chondroitin-4-sulfate	Chondroitin-4-sulfate	IgM
1-B-5	CSPG digested with chondroitinase ABC	ΔDi-0S	IgG1
2-B-6	Same as above	ΔDi-4S	IgG1
3-B-3	Same as above	ΔDi-6S	IgM
2H6	PG from 10-day-old rat brain	Chondroitin sulfate	IgM
473	Extracts of monkey brain	Chondroitin sulfate	IgM
Keratan sulfate *data from Okumura, *et al.*, 1998			
5-D-4	CSPG monomer from human articular cartilage digested with chondroitinase ABC	Keratan sulfate	IgG1
1/14/16H9*	Equine cartilage PG monomer digested with chondroitinase ABC	Keratan sulfate	IgG1
Core protein of proteoglycan			
6-B-6	Human ovarian fibroma	Dermatan sulfate proteoglycan	IgG1
2-B-1	Human yolk sac tumor	Large proteoglycan (versican)	IgG1
HK-102	HSPG from mouse EHS tumor	HS proteoglycan (Perlecan)	IgG2b
1-G-2	CSPG from 10-day-old rat brain	CS proteoglycan (neurocan)	IgG1
6-B-4	PG from 10-day-old rat brain	CS proteoglycan (phosphacan)	IgM

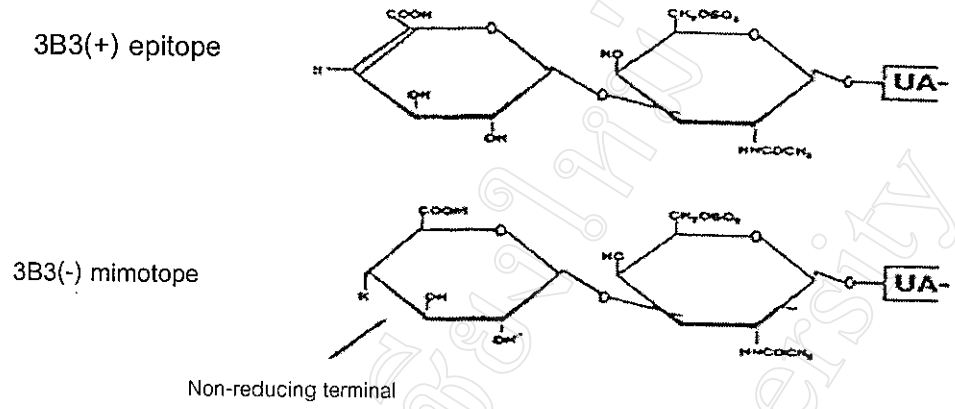

Figure 7 3B3 epitope (top) and mimotope (bottom) (Caterson, *et al.*, 1995)

Equine osteoarthritis

In the equine athletes, acute loss of articular cartilage and bone is a common occurrence (McIlwraith, 1996). The unique properties of the articular cartilage play a crucial role in the efficient performance of the healthy joint. Any aberrations in the composition and structure of an appendicular joint can result in pain and lameness (Todhunter, 1996). Lameness is the most important cause of wastage among horse (McIlwraith, 1996). Joint diseases, which form a major part of the cause of lameness may be occurred from athletic performance, which has contact and pressure through the articular cartilage and induced osteochondral lesion (Brama, *et al.*, 2001) including osteoarthritis.

Osteoarthritis, also called degenerative joint disease, may be defined as a disease of diarthrodial joints comprising destruction of articular cartilage (figure 8) to varying degrees accompanied by subchondral bone sclerosis and marginal osteophyte formation. Synovitis and joint effusion often are associated with the disease. Clinically, the disease is characterized by pain and dysfunction of the effected joints (McIlwraith, 1982; McIlwraith and Vachon, 1988). Considerable attention has been paid in human osteoarthritis to the disease as being an inevitable consequence of aging, and attempts at distinction between degenerative change in articular cartilage associated with aging versus clinical osteoarthritis have been made. The typical equine patient is younger, and it commonly affects young articular cartilage. However, based on pathologic and arthroscopic examinations of older horses' joints, there is no question that there is a pathologic entity of osteoarthritic change in older equine joint, but the clinical significance of that change is poorly defined. The limited perspective of osteoarthritis's being an intrinsic disease of articular cartilage has changed markedly, and enzymatic degradation of articular cartilage is now recognized as a central feature of OA (McIlwraith, 1996)

Morphologic changes associated with osteoarthritis have been well defined, but osteoarthritis is not a simple morphologic event, and there has been a lack of correlation between pathologic changes and their clinical significance (McIlwraith, 1996). The

classification of osteoarthritis in recent years was changed from 5 categories (McIlwraith and Vachon, 1988) in the past to 3 categories (McIlwraith, 1996) as presented in table 3.

Table 3 Classification of osteoarthritis in the horse (McIlwraith, 1996)

Categories	Characteristics
Type 1	Associate with synovitis and capsulitis (common in carpus, fetlock, and distal tarsal and distal interphalageal joints)
Type 2	Associated with (and usually secondary to) other identified injuries or problems including intra-articular fractures, traumatic articular cartilage ligamentous injuries, osteochondrosis, subchondral bone injury and disease, subchondral cystic lesions, septic arthritis, and fragmentation of distal patella
Type 3	Incidental or nonprogressive articular cartilage erosion

Under normal articular conditions, proteoglycan constituents of hyaline cartilage are turned over at a constant rate, and equilibrium exists between synthesis and degradation. In joint diseases, there is loss of the normal balance between synthesis and degradation of the macromolecules in cartilage. In both situations, cartilage proteoglycan fragments are liberated into the synovial fluid, along with fragments of disrupted type II collagen.

There is a predisposition for genetic to develop hindlimb lameness, which is turn associated with radiographic signs of osteoarthritis in the distal tarsus (Axelsson, Björnsdottir *et al.* 2001). Björnsdottir found that there is a moderate heritability (h^2 = 0.4) has been demonstrated for hindlimb lameness including osteoarthritis (Björnsdottir, *et al.*, 2000).

The effect of training at a young age by a professional trainer may also be a risk factor for the development of osteoarthritis in the distal tarsus (Axelsson, *et al.*, 2001) apart from risk factors from racing and riding sports.

Joint disease often starts with inflammation of the tissue that surrounds the joint, called the synovial membrane. The inflamed membrane begins leaking fluid, blood cells and enzymes into the joint, which ignites a process that results in an ongoing cycle of joint destruction and leads to osteoarthritis (figure 9).

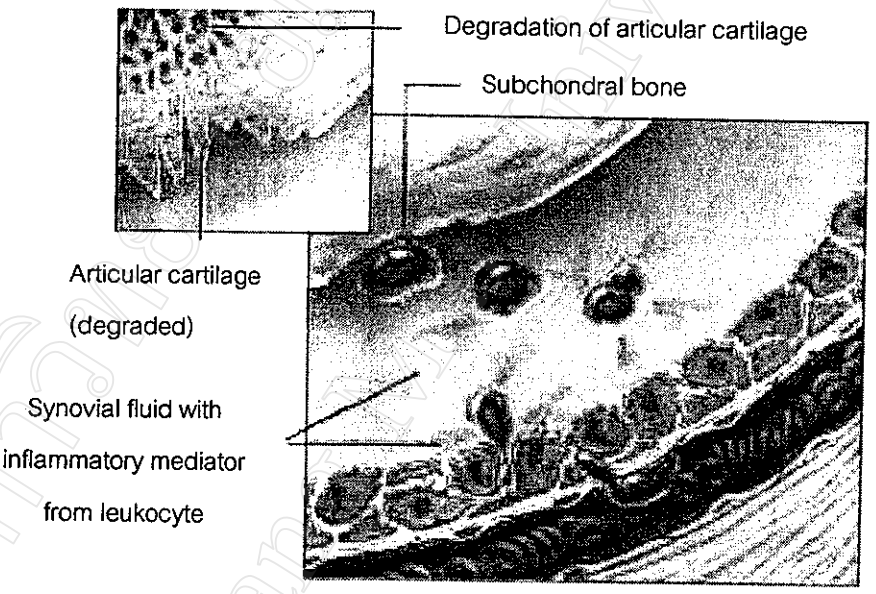

Figure 8 Cartilage degradation (Corporate Country Bayer Group Community, 2001 [Online]. Available: http://www.yourhorseshealth.com/ joint_therapy/struct.html)

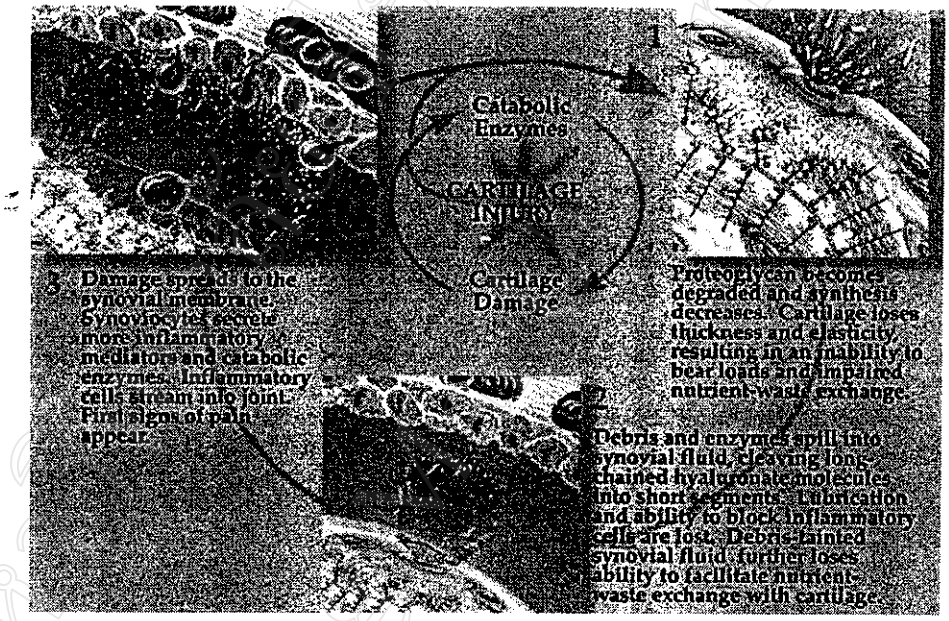

Figure 9 Vicious cycle of joint disease [Online] Available: http://www.luitpold.com/canine/vet/DJD.HTML.

Presumably trauma, instability acute inflammatory disease, or infection in joints causes release of cytokines in excess of their natural inhibitors. This imbalance produces a net loss of proteoglycans and collagen from the matrix and results in mechanical dysfunction that is characteristic of osteoarthritis. The equine situation differs in that synovitis is commonly a primary or at least concomitant event. However, there are considerably more factors involved than simple physical stress injuring the chondrocytes and in turn causing the chondrocytes to release deleterious enzymes (Price, *et al.*, 1992; Gibson, *et al.*, 1996; Ishii, *et al.*, 2002).

Other important factors include observations that whereas the earliest lesion observed may be in the matrix, the cause may be associated with disease of subchondral bone or synovial membrane, which in turn causes degenerative processes in the cartilage. The breakdown products are released from cartilage tissue, and enter to synovial fluid and serum where they are then cleared and excreted. Many factors effected joints are shown in diagram (figure 10). The changing of histologic in induced arthritic cartilage (McIlwraith and Sickle, 1981) and in osteoarthritis cartilage (McIlwraith, 1987) was described.

There are many diagnostic test and also treatment of osteoarthritic as described by McIlwraith (McIlwraith, 1982; McIlwraith and Vachon, 1988). But it is known that radiography is the most important noninvasive imaging technique used to evaluate osteoarthritis in horse. The radiographic changes were described by Widmer (Widmer and Blevins, 1994). However, by the time hallmarks of osteoarthritis are recognizable radiographically, the structural alterations in the articular cartilage are already irreversible. Thus the methods of assessing the extent of articular cartilage destruction *in vivo* before radiographic changes are evident in affected joints may help clinicians select appropriate treatment to prevent development of osteoarthritis (Todhunter, *et al.*, 1997). In recent years, many biochemical markers were tested with the aim of developing a tool for the early detection and monitoring of osteoarthritis not only from cartilage but also other joint

structures (Schmidt-Rohlfing, *et al.*, 2002)(Garnero, *et al.*, 2000). One approach to monitoring articular cartilage destruction is use of ELISA to detect breakdown products such as glycosaminoglycan epitopes, both keratan sulfate and chondroitin sulfate, in body fluid (e.g. synovial fluid, serum, and sometime urine).

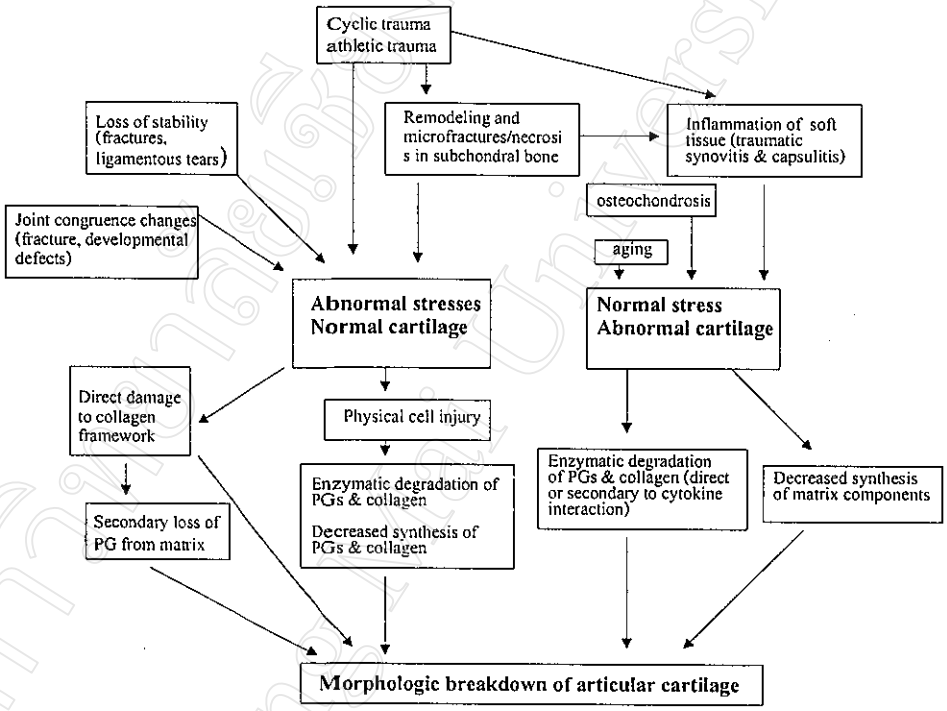

Figure 10 Factors involved in articular cartilage degradation in equine osteoarthritis (McIlwraith, 1996)

OBJECTIVES

1. To investigate the normal value of chondroitin sulfate epitopes (3B3 and WF6 epitopes) and hyaluronan in serum of horses depending on age.
2. To compare the value of chondroitin sulfate epitopes (3B3 and WF6 epitopes) and hyaluronan in serum between normal and osteoarthritic horses.