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COMPARISON OF HEALING OF FULL-THICKNESS CARTILAGE vs. FULL-THICKNESS CARTILAGE AND SUBCHONDRAL BONE DEFECTS IN THE EQUINE THIRD CARPAL BONE

by

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(ABSTRACT)

The quality of repair tissue produced by defects which exposed the subchondral bone plate and those which penetrated the subchondral bone plate to expose the subchondral marrow spaces was compared in the equine third carpal bone. Specimens were examined four and six months after surgery.

Exposure of the subchondral bone plate resulted in a deep layer of fibrocartilage and a superficial layer of fibrous connective tissue. Exposure of the subchondral marrow spaces resulted in a deep layer of hyaline-like cartilage, an intermediate layer of fibrocartilage and a superficial layer of fibrous connective tissue. Degenerative joint disease occurred in all joints. Results were similar between four- and six-month specimens.

Further studies are needed before removal of the subchondral bone plate can be recommended for clinical treatment of full-thickness articular cartilage lesions.

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INTRODUCTION

Traumatic articular cartilage injury is common in the racehorse. Lesions may be confined to the articular cartilage or may be full-thickness defects extending to the subchondral bone. The response of the body to repair these lesions varies with depth, size and location of the defect. Healing usually does not restore normal hyaline cartilage architecture and repair tissue is biomechanically inferior to the original articular surface.

Chondral and osteochondral fragments are factors in the development of degenerative joint disease. Surgical removal of cartilage and osteochondral fragments is accepted as the most successful method to treat articular damage. Controversy exists over the depth of surgical debridement of articular cartilage lesions required to produce the most functional healing for athletic activity. Mature normal articular hyaline cartilage is biomechanically superior to fibrous or fibrocartilaginous repair and is considered the most desirable tissue for resurfacing articular lesions. Numerous studies have demonstrated that when the subchondral bone is exposed, healing is generally more complete than when lesions are confined to cartilage alone.[1,2,3,4,5,6] Work in other species has shown that the subchondral bone marrow spaces are the source of progenitor cells for hyaline cartilage and other types of connective tissue repair, and that proliferation of these cells occurs only when the spaces are exposed.[2,3,5]

The purpose of this study was to compare the quality of repair tissue produced by defects which simply exposed the subchondral bone plate and those which penetrated the subchondral bone plate to expose the marrow spaces. The hyaline tissue content and histologic quality of repair by tissue healing of full-thickness defects was hypothesized to be improved by complete removal of the subchondral bone plate.

LITERATURE REVIEW

Types of Cartilage

Cartilage is a type of semi-rigid connective tissue found in many areas of the mammalian body during growth and maturity. Cartilage functions to provide support for soft tissues, to facilitate joint movement and is the substrate for endochondral ossification of long bones. The basic structure of cartilage consists of a large amount of intercellular matrix with special cavities known as lacunae containing the cartilage cells (chondrocytes). Cartilage is avascular, and nourishment must be derived from diffusion of nutrients from capillaries in periarticular tissues or from synovial fluid in joints. Cartilage has no lymphatic or nerve supply.[7]

There are three types of cartilage, differing primarily in collagen and matrix composition.[7] Hyaline cartilage is the most abundant type and may be articular or non-articular in location. Elastic cartilage is present in a few areas related to the auditory system and in some of the laryngeal cartilages; it is frequently contiguous with hyaline cartilage. The matrix of elastic cartilage contains elastin fibers in addition to collagen fibers. Fibrocartilage is considered an intermediate tissue between hyaline cartilage and dense fibrous connective tissue and is always contiguous with dense connective tissue. The fibrocartilage matrix is dense and contains coarse collagen fibers. Fibrocartilage is normally found in intervertebral discs, menisci, the symphysis pubis and in some ligamentous and capsular attachments to bone.

Hyaline Cartilage Histology and Ultrastructure

Hyaline cartilage in skeletally mature animals is found in the respiratory passages from the nasal septum through the bronchi, costal cartilages, pinnae and joint surfaces. Articular hyaline cartilage differs from that in non-articular sites in the relative proportions and anatomic organization of hyaluronic acid, collagen and proteoglycans. Non-articular hyaline cartilage is covered by perichondrium, a layer of dense connective tissue that assists in growth and maintenance of the cartilage. Perichondrium provides a major contribution to repair of non-articular hyaline lesions via cells that resemble fibroblasts.[7,8] The following discussion pertains to articular hyaline cartilage although many characteristics are shared with the non-articular forms.

Grossly the surface of articular cartilage appears smooth, but microscopic sections demonstrate that the surface is actually irregular with depressions and raised areas creating an undulating surface.[9,10,11,12] Cartilage thickness varies between joints and between locations in the same joint in relation to the amount of weight-bearing.[9,13,14,15]

The majority of hyaline cartilage content is intercellular matrix with few interspersed chondrocytes.[16,17,18,19] The chondrocytes inhabit spaces (lacunae) although the cavitary appearance of the lacunae is actually an artifact of staining for light microscopy.[20] Electron microscopy identifies a territorial zone or capsule immediately adjacent to the cells. The capsule is characterized histochemically as containing primarily water and glycosaminoglycan with little collagen.[21,22,23,24,18] Beyond the capsule is a perimeter of very fine collagen fibers which exists only around the capsule.[25] Surrounding the fine fiber network is a dense area of regular collagen fibers oriented concentrically around the cells termed the perilacunar rim.[26] The arrangement of chondrocytes, collagen and matrix varies within the cartilage from the surface to the subchondral bone and has been divided into anatomically distinct zones.[27,28] Four zones of cartilage are recognized: tangential, transitional, radiate and calcified zones.

The tangential zone is the most superficial layer and is covered by a very thin (three microns) network of fine filaments called the lamina splendens.[29] Boundary protein adheres to the lamina splendens, providing some lubrication of the cartilage surface.[30,31] Beneath the lamina splendens are elongated, flattened chondrocytes which are histologically similar to fibroblasts.[31,32,33] Synthesis of glycosaminoglycan by superficial chondrocytes is minimal, and collagen is the predominant product of the cell. Superficial intercellular matrix is therefore proportionally high in collagen and very low in proteoglycan.[31,34,35] Collagen bundles in the tangential zone are aligned parallel to the surface.[24,25,28,31,36,37,38]

Just below the tangential zone is the intermediate or transitional zone. Chondrocytes are rounded or ovoid and are interspersed singly or paired throughout the matrix.[27,28] Ultrastructure of chondrocytes is indicative of active protein synthesis and includes well-developed rough endoplasmic reticulum, Golgi apparatus, mitochondria and microtubules.[31,39] The collagen fibers in the interterritorial zones (between cells) are oriented randomly although they tend to align at 45° and 135° angles among themselves.[38] Small diameter collagen fibrils and proteoglycans attach to the collagen fibers to form a structural network.

The radiate zone contains chondrocytes arranged in short vertical columns of five to seven cells per column.[20] The cells, smaller than those of the transitional zone, contain a calcified material in their vesicles.[20] Large bundles of collagen fibers perpendicular to the articular surface separate the cell columns and are anchored in the underlying calcified zone.[22,40]

The calcified cartilage zone contains hydroxyapatite salts throughout its matrix.[40,41,42,43] Many chondrocytes are in various stages of organelle degeneration.[18,19,31,39] and have minimal DNA synthesis as demonstrated by their limited uptake of tritiated cytidine.[44] Remodelling occurs actively in this zone through vascular invasion and endochondral ossification.[45]

The tide mark is an undulating, histologically apparent line that separates the radial and calcified cartilage zones.[28] The line appears basophilic when stained with hematoxylin and eosin stain but does not appear with some other stains.[40,46,47,48] The exact nature of the tide mark has not been determined, and proteoglycan or other known basophilic materials have not been demonstrated.[40] One hypothesis is that the line results from a change of direction of collagen fibers as they pass into the calcified zone, and the directional change could give extra support against shear stresses.[49] The tide mark might serve as a "tethering site" for collagen fibers as they cross into the calcified zone,[20] and it may replicate several times with age.[40,45]

The subchondral bone plate is contiguous with the deep regions of the calcified zone through an undulating junction which probably gives some protection from shear stresses.[43] The subchondral plate is typical lamellar bone with haversian systems which are oriented transversely and parallel with the joint surface.[20] Beneath the cortical bone are cancellous marrow spaces of the epiphyseal bone.

Physical Properties of Normal Articular Cartilage

Water accounts for up to 75% of the wet weight of adult articular cartilage with the remainder attributed to cells, collagen, proteoglycan and other matrix components.[50] On a dry weight basis collagen accounts for 60% and proteoglycan for 30% to 35% of articular cartilage;[51,52] ash content has been calculated at 5% to 6%.[52] The remaining dry weight is due to small amounts of lipid, lysozyme, hyaluronate and various glycoproteins.

The precise method of water binding in cartilage has not been determined, but current evidence suggests that water may form a complex gel with both collagen and proteoglycan.[53,54,55,56] Gel formation would allow water to freely exchange with the water of the synovial fluid, and the physical principles of osmotic solutions would still apply.[35,57,58] Hydration of cartilage is assisted by the polyanionic charges of matrix proteoglycan which attracts a large hydration shell.[36,59] This large quantity of water creates a large osmotic swelling pressure which is counteracted by the collagen fibers.[60] Water content increases from the superficial to the radiate zone and diminishes in the calcified zone of cartilage.[50]

Proteoglycan structure has been characterized as a linear protein with polysaccharide complexes referred to as glycosaminoglycans attached directly and at right angles to the protein core.[33,61] Glycosaminoglycans (GAG) are long-chain repeating disaccharide units. Three GAG molecules are recognized in the proteoglycans of articular cartilage: chondroitin 4-sulfate, chondroitin 6-sulfate and keratan sulfate.[62,63] Chondroitin 4-sulfate is primarily found in skeletally immature animals and decreases with age.[64] Chondroitin 6-sulfate is the most prevalent GAG in adult articular cartilage with peak levels in the radiate zone.[50] Carboxyl and sulfate radicals give the GAGs a polyanionic structure which tends to repel other GAGs and attract water, creating compressive stiffness in the cartilage.[65]

The proteoglycan subunit is attached via a link protein to hyaluronic acid, which is a glycosaminoglycan existing only in the aggregate formed with the many attached proteoglycans.[66,67,68] The proteoglycan aggregates are organized in the collagen framework such that the concentration of aggregates is lowest in the superficial layers of articular cartilage and increases in the deeper layers.[35]

In addition to the large proteoglycan aggregates, other types of noncollagenous matrix proteins have been identified. Two small chondroitin sulfate proteoglycans are recognized and comprise 1% to 2% of the total proteoglycan mass. One of these small proteoglycans (PG-S2) binds to collagen and appears to modulate collagen fibril formation.[60] Fibromodulin is another protein which modulates collagen fibrillogenesis. Other cartilage matrix proteins which function to bind collagen, anchor collagen to cells, interact with proteoglycans and attach chondrocytes have been characterized.[60]

The tropocollagen of articular hyaline cartilage consists of three identical alpha(1)-helical chains and is referred to as Type 2 collagen.[69,70,71] Type 2 collagen contains significantly higher amounts of hydroxylysine and carbohydrate than Type 1 collagen.[71] Collagen content is highest in superficial layers of articular cartilage and diminishes with depth.[35] Cross-linking via covalent bonds between collagen and proteoglycan aggregates, physical entrapment of the proteoglycans in the collagen framework and the orientation of collagen gives different biomechanical properties to cartilage which vary according to depth from the articular surface.[72]

Physiologic Properties of Normal Articular Cartilage

Articular chondrocytes are metabolically active cells despite the avascular structure of articular cartilage. Synthesis of matrix proteins, glycosaminoglycans and collagen and assembly of these components into macromolecules occur intracellularly. Chondrocytes primarily use anaerobic glycolysis to produce energy for cell processes.[45,73,74] Oxygen tension is postulated to provide a control mechanism for the rate of cell metabolism.[45] Oxygen and other nutrients are supplied by the synovial fluid which diffuses under pressure from opposing cartilage surfaces through the fluid fraction of cartilage matrix.[75]

The rate of loss and replacement of matrix proteoglycan indicates that there may be two "fractions" of proteoglycan, one which has a rapid turnover rate (fast fraction) and another with a slower turnover rate (slow fraction).[76] Replacement occurs uniformly throughout the cartilage, suggesting an enzymatic remodeling of proteoglycan rather than attrition of only superficial proteoglycans.[20] The source of enzymes is not known, but all of the enzymes which degrade proteoglycans have been identified in articular cartilage with the exception of hyaluronidase which has been identified in the synovial membrane.[50] The varying fast and slow turnover rates further suggest that proteoglycan structure may be heterogeneous.[20]

Lubrication of articular cartilage occurs via two systems, boundary lubrication and hydrostatic lubrication.[77,78] Boundary lubrication is a low-load friction reduction system provided by a thin layer of glycoprotein which covers and adheres to the cartilage surface.[77,78] Hydrostatic lubrication, also known as "squeeze film" lubrication, occurs under axial loading which causes water to extrude from the cartilage matrix.[78] The water forms an interface between the cartilage surfaces during elastic deformation of the cartilage and is contained by the undulations of the surfaces.

Biomechanical properties of cartilage are principally conferred by the arrangement and concentration of collagen and proteoglycan aggregates which vary according to depth from the articular surface. In the superficial layer, the proportion of collagen to proteoglycans is high and collagen fibers are oriented tangentially to the surface. The tangential arrangement maximizes resistance to tensile forces from the opposing articular surface.[79] In the deeper layers, proteoglycan content is relatively higher than collagen. Hydration pressure of the ground substance provides compressive stiffness to the cartilage.[65] The tide mark represents the junction of two structurally and biomechanically different materials and therefore is an area of susceptibility to shearing stress.[80]

Properties of Fibrocartilage

Fibrocartilage is closely associated with dense connective tissue and has characteristics intermediate between hyaline cartilage and dense fibrous connective tissue. The chondrocytes of fibrocartilage are similar to hyaline chondrocytes and are frequently found in long cell columns. Type 1 collagen fibers are abundant and may form irregular or parallel bundles between the chondrocyte columns. The proteoglycan matrix is identical to that of hyaline cartilage but exists in relatively less quantity due to the high concentration of collagen fibers. Perichondrium is not associated with this type of cartilage.[7]

Healing of Cartilage Defects: Historical Perspective

The fate of articular cartilage lesions has been debated in the literature for over two centuries. Hunter in 1743 wrote "From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing and that when once destroyed it is not repaired."[81] The opinion that articular hyaline cartilage possesses limited or no ability to repair lesions persisted until 1851 when Redfern demonstrated that articular cartilage could heal by the formation of fibrous tissue across lacerations created in the cartilage.[82] He believed the origin of this fibrous tissue to be the intracellular contents of chondrocytes adjacent to the lesions which he observed to be enlarged and rounded. Chondrocytes away from the lesions were of normal size, therefore he believed the cells adjacent to the lesion were responding to the stimulus of injury.

Gurlt (1853) found that articular cartilage defects fill with fibrous connective tissue which could change over time to become a mixture of fibrocartilage and fibrous tissue, but a pure hyaline cartilage structure was never achieved.[83] Ogston (1875) observed that articular cartilage could not proliferate laterally but could only grow toward the articular surface, therefore healing of defects could not occur by centripetal cartilage growth.[84]

Following Redfern's observations, many investigators published reports on the ability of hyaline cartilage to repair injury. Early studies were of experimental defects in the pinna and costal cartilages.[8,85,86,87,88] Non-articular hyaline cartilage is enveloped in perichondrium except at its attachment to bone, and repair tissue is universally observed to originate from the perichondrium. Articular hyaline cartilage is not covered by perichondrium, therefore experiments with non-articular hyaline cartilage do not truly represent the healing capability of articular cartilage. Additionally, the normal architecture of articular cartilage is different from non-articular hyaline cartilage, and joint surfaces are subjected to a variety of biomechanical forces which affect healing.

Effects of Mechanical Trauma on Articular Cartilage

Traumatic lesions to cartilage may be classified according to lesion size, location and depth. Of these, lesion depth appears to be most important in defining the type of healing response which occurs in the injured tissue. Lesion size and location modify the healing response primarily due to mechanical factors imposed on the affected area.

Lesion depth is traditionally referenced as partial-thickness or full-thickness. Partial-thickness defects are superficial and may involve the articular cartilage down to the tide mark.[80] Full-thickness lesions are those which penetrate the tide mark and may involve the subchondral bone plate. Some authors refer to partial-thickness lesions as those which do not involve subchondral bone and full-thickness lesions as those which expose or enter subchondral bone.[20]

Lesions which do not completely penetrate the calcified cartilage zone are confined to avascular tissue with no access to inflammatory and repair cells.[20] Following injury a brief phase of necrosis occurs along the margins of the injured area, and ghost cells may be seen in adjacent lacunae. This phase is followed by increased mitotic activity in adjacent cartilage and a transient increase in matrix synthesis.[89] This period of increased chondrocyte replication and matrix production lasts only one week and metabolic activity then returns to normal levels.[90] The result is a slight increase in chondrocyte number and matrix which is insufficient for complete repair.[20] Partial-thickness lesion response is independent of lesion diameter or orientation.[20]

Lesions which penetrate the calcified cartilage zone to involve the subchondral bone plate and expose the vasculature of the bone elicit a more typical inflammatory healing response.[4,14,91,92,93,94,95,96,97,98] The defect fills with blood and a fibrin clot is formed, trapping red blood cells, white blood cells, platelets and bone marrow cells.[4] Undifferentiated marrow cells begin to resemble fibroblasts[5] concurrently with vascular invasion of the clot, and granulation tissue is formed.[5,89,99] The tissue becomes less vascular over time.[4,61,91] New bone formation is rapid in the areas which contact injured osseous tissue but the process stops at the original margin of the calcified cartilage and bone.[20] The fibrous repair tissue in the cartilage defect undergoes hyalinization to varying degrees to form fibrocartilage.[91,94] As seen in partial-thickness lesions, chondrocytes adjacent to the defect display a brief period of mitosis and increased matrix production; this response contributes little to the overall repair.[89] While the ability of full-thickness lesions to repair with hyaline-like tissue is recognized, the long-term fate of the repair tissue is seldom mature hyaline cartilage. Most reports confirm that degeneration of the replacement tissue, especially the superficial layers, results in fibrous or fibrocartilaginous tissue filling the defects. Mitchell and Shepard showed that the tangential orientation of superficial collagen fibers is not reestablished, and architecture of other layers is never normal following full-thickness injury.[97] Lesion diameter affects the final repair of deep lesions with the smaller diameter lesions showing the most complete repair.[94] Continuous passive motion has been shown to have a beneficial effect on both rate and quantity of hyaline cartilage repair of full-thickness lesions in rabbits.[100]

In an experiment related to cartilage healing, Hall (1969) resected the lateral femoral condyle in rats and demonstrated degeneration of the opposing tibial articular cartilage.[101] Presumably, loss of contact with the normally opposed surface resulted in loss of superficial chondrocytes, surface flaking, fibrillation and loss of cartilage down to subchondral bone. Motion was allowed postoperatively so immobility was not a factor in the cartilage degeneration which resulted. Since inflammation was not controlled, the study did not prove why the cartilage underwent degenerative change but agreed with other investigations that altered diffusion of nutrients into cartilage is a likely mechanism.

Healing of Experimentally Created Partial-Thickness Lesions

In 1878 Tizzoni created incisions in articular cartilage and observed granular degeneration of chondrocytes on the margin of the incisions with an adjacent zone of chondrocyte enlargement.[102] He observed that mesenchymal cells in the marrow cavities underwent metaplastic change to cells resembling chondrocytes when the incisions extended into the subchondral bone. Seggel (1904) observed the response of chondrocytes adjacent to shallow, linear incisions which he further characterized to include formation of clusters of chondrocytes known as chondrones and new hyaline matrix production.[103] However, Seggel found that five months after creation of these defects the chondrocyte response was no longer present, and the defects remained with minimal evidence of healing.

Haebler (1925) made superficial defects in canine articular cartilage and showed no healing at 300 days after surgery.[104] He stated that this result proved that hyaline cartilage had no inherent ability to repair itself, and only when the subchondral bone was traumatized could the defect fill with fibrous tissue.

Shands (1931) was able to demonstrate regenerative capability in articular hyaline cartilage in agreement with other investigations.[2] In dogs, lesions were created only through the superficial cartilage layers in weightbearing and non-weightbearing areas of the femorotibial, femoropatellar, humeroradial and carpal joints. In response to the presence of superficial lesions, chondrocytes adjacent to the lesion were enlarged, deeply stained and occasionally multinucleated. As further evidence of attempted regeneration, the cell columns of the adjacent cartilage were tilted toward the lesion although the matrix in this area stained poorly. Fibrillation of the incised edges was frequently seen. The period of greatest regenerative change was between four and eight weeks, at which time the study of superficial lesions was ended.

Bennett, Bauer and Maddock (1932) created superficial lesions in weightbearing and non-weightbearing areas of the adult canine femoropatellar joint.[3] The accidental postoperative occurrence of persistent medial patellar displacement produced marked joint pathology in many joints. They were, however, able to observe proliferation of chondrocytes adjacent to superficial lesions and noted that this proliferation was more pronounced in lesions of the weightbearing portion of the femoral condyle than in the non-weightbearing area of the trochlear groove. Grossly, the defects remained plainly visible, and histologic repair of these lesions was not complete after twenty-eight weeks. Bennett and Bauer in 1935 created partial-thickness lesions in the patellar groove of skeletally immature dogs (prior to physeal closure) and concluded that age did not affect rate or manner of repair.[105]

Calandruccio and Gilmer (1962) created superficial lesions one-third to one-half of the thickness of the cartilage in the articular surface of the femoral condyles and the patellar ridges in juvenile dogs.[91] Lesion diameter was not specified. A zone of cell death was noted in the cartilage immediately adjacent to the defects. Chondrone formation without concurrent increase in matrix formation was observed in the cartilage adjacent to the zone of cell death. A shelf of matrix protruded into the defects from the superficial layers of adjacent cartilage as observed by Shands[2] and was termed "matrix flow". Matrix flow was seen where the lesion walls approached a vertical orientation, in apparent support of mechanical pressure producing this phenomenon. Surface bridging of the defects by chondrocytes in a collagenous stroma occurred in some two to three week specimens. The source of this bridging tissue was the superficial layer of adjacent original cartilage. The number of blood vessels beneath the superficial defects increased in several five to eight week specimens. These intracartilaginous blood vessels, normally present in immature hyaline cartilage, may have contributed to healing. New hyaline cartilage was a consistent finding in the base of the superficial defects. The investigators postulated that granulation tissue might inhibit hyaline cartilage formation since granulation tissue was not present in the depths of the defects and hyaline cartilage repair occurred in these areas. The superficial layer of articular cartilage was the proposed source of chondrocytes in all articular cartilage, a different opinion from earlier reports which implied that the intermediate layer of cartilage was the source of chondrocytes. This conclusion was reached based upon the presence of hyaline repair tissue which was continuous with the adjacent superficial hyaline cartilage extending across the defect.

In 1966 DePalma, McKeever and Subin cited the need for an objective study of articular cartilage healing, suggesting the different opinions in the literature were due in part to subjective interpretation of histologic results.[5] The radioactive nucleotide tritiated thymidine was used to label and identify the presence of DNA synthesis and thereby identify regenerating tissues. Autoradiography of labelled tissue was employed to identify which cells in the articular cartilage were participating in its regeneration. Partial-thickness lesions were created in weightbearing and non-weightbearing areas of the canine femorotibial joints, and samples were collected at weekly and monthly intervals until 66 weeks after surgery. A thin rim of tissue projected slightly into the defects from the adjacent superficial layer of cartilage. There was no repair in any histologic sections at any period of the study. Autoradiographic analysis did not show significant regeneration in the partial-thickness lesions. The authors concluded that adjacent articular cartilage does not contribute to partial-thickness cartilage lesion healing and such lesions have no evidence of repair 66 weeks after creation of the defects.

Ultrastructural studies of superficial cartilage defects in rabbits defined increased metabolic activity in adjacent cartilage cells.[106] However, six months after creation of the lesions a thin layer of new matrix and collagen fibrils was present across the surface of the defects. Matrix "flow formations" were identified which covered the depths of the lesions three to six months after creating superficial lesions.[107] Weight-bearing supposedly caused the matrix flow, and matrix flow was the only mechanism by which adjacent cartilage partially filled superficial lesions.

Ghadially, Thomas, Oryschak and Lalonde (1977) followed superficial lesions for two years to examine the long-term fate of repair.[11] Flow formations were not present at 18 and 24 months after surgery, and the surface of the repair tissue had a pitted appearance suggestive of surface wear by the opposing articular surface without ability of the damaged cartilage to replace the lost matrix. The long-term result was that superficial defects did not heal.

Healing of Experimentally Created Full-Thickness Lesions

The healing response in lesions extending into subchondral bone was examined by several early investigators. Fasoli (1905) showed complete hyaline repair of linear incisions when the defects extended to subchondral bone.[108] Rimann (1905) created deep defects in articular cartilage and found that at 31 days the defects were filled with a cellular tissue arising from the marrow which eventually underwent metaplasia to form hyaline cartilage.[109] Ciocola (1921) made superficial tangential incisions into articular cartilage and found no healing after two months.[110] Defects extending into subchondral bone were repaired with connective tissue which underwent transformation to hyaline cartilage. Fisher (1922) contrasted healing according to lesion depth and location, showing that six weeks after creating superficial cartilage defects no repair occurred unless the defect had been made near the perichondrial margin of the joint.[1] He observed that healing of such lesions did occur when subchondral bone was accidentally exposed during creation of the superficial cartilage defects in areas away from the articular margins of the joint. Fisher's work, however, was not intentionally designed to distinguish healing by contrasting lesion depth.

In 1924 Ito outlined the sequence of healing of defects extending into the subchondral bone in rats.[111] One week after creating the defects, fibrous tissue filled the defects. Fibrocartilage had appeared after two weeks, and in three weeks, islands of new hyaline cartilage were present in the deep layer of repair tissue. The source of repair tissue was the cancellous marrow spaces. In a similar experimental model, Key (1931) observed osteogenic cells in the marrow spaces to proliferate as part of the repair process in full-thickness defects.[96]

Shands (1931) was among the earliest to attempt to experimentally distinguish variations in healing according to lesion depth.[2] Lesions were created through to calcified cartilage or subchondral bone in dogs. Lesions were created in different joints, in non-weightbearing and weightbearing locations in the various joints, and in areas with and without perichondrial covering. The lesions made into calcified cartilage had a similar and slightly more pronounced chondrocytic response than the superficial lesions and included production of new hyaline matrix. Some of these lesions filled partially with connective tissue and cartilage cells. Shands believed the cartilage cells had been extruded from the cut edge of the cartilage. The defects extending to subchondral bone were filled initially with fibrin, then granulation tissue followed by fibrocartilage and areas of new hyaline cartilage. The edges of adjacent matrix were bent over the defects in a "hooklike" fashion. Repair tissue was observed to be proliferating from the exposed marrow spaces. In addition, evidence of repair of the injured subchondral bone was observed in one twelve week specimen, although most defects displayed some fragmentation of the injured subchondral bone. The conclusions were that the best regeneration of hyaline cartilage was seen when defects involved subchondral bone, and this result did not appear to vary between joints or between weightbearing and non-weightbearing areas of the canine stifle joint.

Bennett, Bauer and Maddock (1932) characterized the repair of non-weightbearing patellar groove lesions in mature dogs. Subchondral bone was removed to a depth of 2 mm with its overlying cartilage but lesion diameter was not specified.[3] The repair of the full-thickness lesions was observed between four and twenty-eight weeks. Fibrous connective tissue extended into the defects from the marrow spaces. The subchondral bone plate had begun healing by twelve weeks after surgery. The calcified zone of cartilage was reforming at the lateral margins of the defect in some specimens after twenty weeks. Above the reforming bone plate was a dense, avascular layer of connective tissue. Hyaline-like tissue was seen superficially after twenty weeks. Fusion of repair tissue with adjacent cartilage was observed at the lesion margins. The conclusion was that hyaline cartilage has a limited ability to regenerate. Additionally, the majority of healing is provided by mesenchymal cells from the subchondral bone which form fibrous tissue that transforms into fibrocartilage and isolated areas of hyaline cartilage. Another conclusion was that the presence of small superficial or full-thickness lesions did not seem to cause significant joint pathology.

In 1962 Calandruccio and Gilmer published results of articular experiments in puppies.[91] One procedure involved removal of a 4-mm diameter cylinder of cartilage and underlying subchondral bone from the weightbearing surface of a femoral condyle. Chondrocyte attrition and decreased staining in the adjacent cartilage was observed two weeks postoperatively. Superficial adjacent cartilage matrix appeared to be "flowing" into the defects (matrix flow). Possible explanations for matrix flow included decreased cohesiveness of the matrix following chondrocyte death and mechanical pressure from the opposing joint surface forcing the matrix into the defects. Matrix flow appeared consistently at the superficial margins of the defects and may have indicated a more fluid nature to matrix in this region or a greater pressure effect in this area compared to the deeper areas of matrix. The cartilage adjacent to the defects displayed proliferation of superficial chondrocytes and centripetal tilting of cell columns four to six weeks after surgery. These superficial chondrocytes and their surrounding fibrous tissue formed a complete and coherent layer across the defects while fibrous tissue originating from subchondral bone filled the lesion depths. The majority of healing was by granulation tissue from the base of the lesions. The subchondral bone plate began to regenerate by three weeks and eventually reformed at or below the original level; the new bone plate was concave at its surface. Immature hyaline cartilage formed along the walls of the defects between three and eight weeks. The zone of acellular matrix persisted, indicating that the new chondrocytes probably did not originate from the adjacent original hyaline cartilage. The possibility of this adjacent tissue inducing hyaline differentiation in repair tissue was suggested. Hyaline cartilage was not located adjacent to blood vessels deep in the repair tissue. Complete hyaline repair and fusion of new with original hyaline cartilage was seen in three of thirty 4-mm defects (after six, twelve and thirteen weeks). The subchondral bone plate had not completely reformed in these cases. Partial hyaline repair was seen in most of the other defects at six to thirteen weeks, with the remainder of the repair consisting of a mixture of fibrocartilage and fibrous connective tissue. The subchondral bone plate was reestablished in these cases. Full-thickness defects had hyaline cartilage only above the granulation tissue in the depths of the lesions, which was postulated to represent an inhibitory effect of granulation tissue on hyaline cartilage formation.

In 1966 Depalma, McKeever and Subin in their histologic and autoradiographic study of articular cartilage healing created 4-mm diameter full-thickness lesions in weightbearing and non-weightbearing locations of the canine femorotibial joints.[5] Specimens were collected at weekly and monthly intervals until 66 weeks after surgery. Histologically, full-thickness defects in weightbearing areas filled initially with a blood clot which became granulation tissue by four weeks and highly cellular, immature cartilaginous tissue by eight weeks. By 16 weeks all defects were filled completely with hyaline cartilage, and at 65 to 66 weeks the subchondral bone plate had completely reformed and the hyaline cartilage had matured. Full-thickness defects in non-weightbearing areas differed from weightbearing areas in that hyaline regeneration did not fully develop until 32 weeks and there was less hyaline tissue content. Autoradiographic analysis was performed using tritiated thymidine to identify DNA synthesis and thereby identify actively regenerating tissues. Increased labelling was present in the marrow spaces beneath the lesions two days after surgery. After two weeks, thymidine uptake was equal in the repair tissue and the underlying marrow spaces. Thymidine uptake had slowed by eight weeks and at 32 weeks only the marrow spaces and immature regions of cartilage in the defects had uptake. Significant uptake did not occur in adjacent cartilage at any time during the study. The authors concluded that in full-thickness defects of 4-mm diameter, new immature cartilage fills the lesions by 16 weeks. This hyaline-like tissue arises from subchondral-origin granulation tissue which undergoes metaplastic change to fibrocartilage and subsequently to hyaline cartilage, in agreement with the work of Shands (1931)[2] and Bennett, Bauer and Maddock(1932).[3] Adjacent cartilage plays no significant role in the reparative process of cartilage defects. Furthermore, since healing occurred more rapidly in weightbearing areas, early weightbearing (allowed immediately postoperatively) and motion presumably had a positive effect on healing.

Campbell (1969) presented some observations on clinical cases and unpublished experimental data on full-thickness defects and articular fractures.[4] He stated that almost without exception intra-articular fractures healed with fibrous tissue and fibrocartilage across the fracture gap in the articular cartilage. Hyaline cartilage repair in articular lesions was rare, and when it occurred the hyaline tissue was never mature and never completely filled a lesion. The most complete hyaline repair was seen when lesion size was small or when fractures were anatomically reduced. Campbell believed that granulation tissue is essential for repair of cartilage lesions and is capable of various degrees of metaplasia into fibrocartilage and hyaline cartilage. Because granulation tissue is capable of metaplastic transformation into cartilage, Campbell contradicted Calandruccio's hypothesis[91] that granulation tissue inhibited formation of hyaline cartilage.

Debate continued over the type or types of repair tissue filling full-thickness articular cartilage lesions. Ghadially, Fuller and Kirkaldy-Willis (1971) utilized electron microscopy to examine and characterize repair tissue in surgically-created full-thickness lesions.[112]

Defects 1.5 mm in diameter were made in the weight-bearing surface of the medial femoral condyle of three- to four-month-old rabbits. Samples were collected between four days and six months after surgery. Grossly, a shiny white opaque tissue filled the defects by four weeks, and this appearance did not change noticeably for the remainder of the study. Light microscopy at four days after surgery demonstrated a fibrin clot in the base of the lesion and "lipping" of the superficial adjacent cartilage into the defect. Two zones of repair were distinguishable in the two- and three-week specimens. There was a deep cartilage layer which later appeared to differentiate into a new subchondral bone plate and a superficial zone of cellular fibrous tissue which would replace the defect above subchondral bone. At three months the repair tissue consisted of fewer cells within increased matrix. The six-month specimens closely resembled adjacent cartilage although the normal architecture of articular cartilage was not restored. Repair tissue in these 1.5-mm defects fused well with surrounding cartilage. Toluidine blue staining of repair tissue was always less intense than adjacent cartilage, and the adjacent cartilage stained less intensely than cartilage away from the lesion margins. Electron microscopy identified chondrocyte necrosis as well as chondrone formation in the adjacent cartilage. Chondrones were present as early as four days and persisted throughout the six month period of study; they were metabolically active in the secretion of mucopolysaccharide matrix. There was no migration of cells from adjacent hyaline cartilage into the repair tissue, but some matrix flow occurred and slightly decreased the size of the lesions. Fibroblasts appeared in the repair tissue by one week postoperatively. Cells resembling chondrocytes appeared by four weeks although the matrix formed was dissimilar to hyaline matrix. At three months most cells were clearly chondrocytes and appeared to be producing fibrils, and by six months these cells and their surrounding matrix were identical to hyaline cartilage. The repair tissue was not uniformly hyaline, however, and large areas of matrix without cells were seen. Cellularity decreased toward the surface of the repair tissue, and characteristic zonation of articular cartilage was not reestablished. The changes in repair tissue were summarized as rapid metaplastic change of fibroblasts to chondrocytes with slow and non-uniform matrix production. Matrix production and flow were the main contributions of adjacent cartilage to healing of defects rather than chondrocyte proliferation or migration. The result at six months was hyaline cartilage in some areas of the defect with large areas of acellular matrix in other areas. The repair cartilage was believed to be biomechanically inferior to normal hyaline articular cartilage because of the areas of acellular matrix and lack of characteristic zonation.

Frost in 1979 further distinguished articular cartilage lesions by depth and concluded that the tide mark between the deep layer of non-calcified cartilage and the calcified layer of cartilage is also an important histologic landmark in the healing response.[95] In examining surgically-created defects in the femoral condyles of dogs, he found that removal of articular cartilage down to the deepest portion of columnar chondrocytes (just above the tide mark) led to degeneration of the remaining chondrocytes, sloughing of matrix and exposure of the tide mark. Once the tidemark was exposed, the subchondral bone responded with increased vascularity and cellularity. Between two to eight weeks trabecular bone began "replacing" the calcified cartilage but did not penetrate the tide mark except in places where the tide mark was not intact. In places where the tide mark was not intact, fibrous tissue originating in the subchondral marrow spaces penetrated the tide mark and filled the depths of the lesion. Between eight and sixteen weeks the trabecular bone growth penetrated intact portions of the tide mark allowing further fibrous tissue ingrowth; this fibrous tissue eventually underwent metaplasia to fibrocartilage. Frost concluded that when the tidemark was disrupted or exposed by trauma the subchondral bone mounted a vascular response which resulted in fibrocartilage healing of the lesion.

Lesions which penetrate the tide mark but do not involve the subchondral bone were examined in a study by Cheung et al (1978) in the rabbit femur.[92] The typical period of increased metabolic activity in adjacent cartilage to the defects was observed and was also noted at the base of the defects. The chondrocyte response was more pronounced than that seen in superficial defects, and fibrous tissue lined but did not fill the defects. This response was intermediate to superficial and deep defects, and the difference was theoretically due to either a reparative response in the calcified zone or perhaps an unknown stimulus affecting chondrocytes of different zones in different ways. Similar to superficial defects, however, only Type 2 collagen was produced by the chondrocytes.

Healing of Experimentally-Created Full-Thickness Lesions in the Horse

The first report of articular cartilage healing following creation of experimental defects in the horse was by Riddle in 1970.[6] The carpus was selected for study because of the high incidence of traumatic carpal lesions in racehorses. The proximal surface of the third carpal bone was used in both carpi of each animal. Also, the radial facet of the third carpal bone in one limb and the intermediate facet of the third carpal bone in the opposite limb were utilized. Lesions measured 1.5 sq.cm. in horses and 1.0 sq.cm. in ponies and were intended to be full-thickness in all cases. Histologically, full-thickness lesions had not been created in some areas, leaving the tide mark intact at these locations. None of these partial-thickness areas had healed at the eight-month conclusion of the study. Full-thickness defects were covered by granulation tissue at one month; the granulation tissue originated in the subchondral marrow spaces and in the perichondrium at the articular cartilage margin. The granulation tissue differentiated into fibrocartilage by two months, with cells resembling chondrocytes and matrix which stained positively for proteoglycans. Six months after surgery the repair tissue was grossly white and firm; histologically the tissue resembled hyaline cartilage. Microradiographs of the defects identified enlarged marrow spaces with osteoclasts present until six months after surgery when the spaces and bone plate appeared normal. Riddle also described the appearance of lesions on the opposing carpal bones which were first seen four months after surgery. These lesions were deficient in proteoglycan, and their surface was thinned and roughened. Another observation was the presence of osteophytes on the dorsal distal aspect of the radial carpal bones at eight months. The osteophytes were not located in areas covered by synovial membrane, and Riddle theorized that, since they occurred only in direct opposition to the surgical defects, mechanical factors had likely resulted in ossification of marginal articular cartilage.

Convery, Akeson and Keown (1972) studied full-thickness defects of varying diameters in the horse to determine whether lesion diameter was related to the quality of repair.[94] The defects were 3, 9, 15, and 21-mm in diameter, and all but the 3-mm defects were made in the weight-bearing area of the medial femoral condyle. In addition, the subchondral bone plate was completely removed to expose the marrow spaces. Only the 3-mm defects had complete repair on gross inspection, and nine months were required to achieve this degree of healing. Histology showed the repair tissue to be a mixture of fibrous tissue and fibrocartilage at three and six months and hypercellular cartilage at nine months. The larger defects filled partially with tissue originating in the marrow spaces. This tissue was characterized as a mixture of fibrous tissue, fibrocartilage and hypercellular cartilage. The subchondral bone plate was not reestablished in any large defect. Glycosaminoglycan content gradually increased over the nine month study period but did not achieve control values. Total collagen content reached control values by the ninth month. The study confirmed a relationship between increasing lesion diameter and inferior healing quality.

Grant (1975) created 4-mm and 8-mm full-thickness defects at several locations in the carpus (distal radius, distal radiocarpal bone, proximal third carpal bone); all defects were located at the dorsal joint margins and were examined up to 67 weeks after surgery.[113] Gross examination revealed two types of repair tissue. Firm, white tissue usually filled the side of the defect away from the dorsal edge of the bone and firm, cream-colored tissue associated with a synovial adhesion filled the dorsal edge of the defect. Most lesions were associated with a synovial adhesion at the dorsal edge of the lesion. Microscopically, no defects were filled with mature hyaline cartilage. Most were filled deeply with immature hyaline cartilage and superficially with fibrocartilage, and the fibrocartilage content actually increased after 47 weeks. Histologically, the firm, white repair tissue resembled immature hyaline cartilage, and the cream-colored repair tissue was a mixture of fibrocartilage and fibrous connective tissue. Grant observed good attachment of the deeper hyaline repair tissue to the subchondral bone but rarely observed complete attachment of repair tissue to the adjacent margins of normal hyaline cartilage. Postoperative radiographic examination at seven weeks showed new bone proliferation at the distal radiocarpal joint capsule attachment in 6 of the 24 operated joints, but there was minimal proliferation at the twenty week examination. Osteophytes formed in five carpi at the distal radius and distal radial carpal bone defects. The study concluded that during surgical treatment of osteochondral carpal lesions the lesion base should be curetted to expose bleeding subchondral bone of firm consistency so that some form of hyaline repair can occur. Also, since repair tissue had minimal change after four months, little benefit was felt to be derived from postoperative rest periods longer than four months.

Hurtig, Fretz, Doige and Schnurr (1988) studied cartilage healing of full-thickness defects of different diameter in both weightbearing and non-weightbearing areas of the femoropatellar, radiocarpal and intercarpal joints.[114] Lesion diameter was small (5mm) or large (15mm). Femoropatellar defects were made in the lateral trochlea in an area contacted by the patella and on the axial aspect of the lateral trochlea away from patellar contact. Weightbearing lesions in the carpus were created in the proximal radial carpal and third carpal bones 1.5-cm from the dorsal rim of each bone. Non-weightbearing carpal lesions were created on the rounded dorsal rim of the radial and third carpal bones. All lesions extended to bleeding subchondral bone. Specimens were examined at 1, 2.5, 4, 5 and 9 months. Healing was least effective on the dorsal rim of the third carpal bone. Dorsal rim lesions were associated with synovial adhesions and perichondrial reaction which presumably interfered with healing, and these lesions actually enlarged in size over the course of the study. The perichondrial reaction was associated with osteoclastic resorption of subchondral bone and attrition of perilesional hyaline cartilage. Articular surface lesions away from the dorsal rim were not affected by synovial adhesions and perichondrial reaction. Matrix flow was observed in all lesions and was especially prominent in stifle lesions, contributing to reduction in lesion size of 50% to 90% in stifle lesions in the first 2.5 months. The quality of repair in large defects was considered good at 2.5 months but cleft formation between the repair tissue and underlying bone occurred by five months postoperatively. Biomechanical forces from opposing articular surfaces were postulated to have caused the cleft formation beginning at defect margins where attachment of repair tissue and adjacent cartilage was generally incomplete. It was speculated that as lesion size increases, the impingement on the repair tissue from opposing surfaces theoretically increases and predisposes larger lesions to disruption of repair tissue. Failure of repair was also attributed to incomplete restoration of matrix proteoglycan which structurally weakens the repair tissue. Although proteoglycan content in repair tissue increased during the nine month study period, it was always markedly decreased from adjacent normal articular cartilage. A one year period of recuperation was recommended following surgical treatment of large cartilage defects to maximize attachment of repair tissue to subchondral bone and to promote maximal proteoglycan content before subjecting the tissue to athletic stresses.

Adjunctive Surgical Procedures in the Treatment of Articular Cartilage Lesions: Osteostixis and Spongialization

Surgeons have debated appropriate treatment of articular cartilage lesions which would restore hyaline cartilage or a biomechanically comparable replacement tissue. Pridie (1959)[115] and later Insall (1974)[116] proposed that multiple drill holes through exposed subchondral bone into the underlying marrow spaces would aid fibrocartilaginous resurfacing of osteoarthritic lesions in the human knee. Mitchell and Shepard (1976) performed the described subchondral drilling in rabbits.[97] Two to four months after drilling the holes hyaline-like cartilaginous tissue filled the drill holes and the full-thickness articular surface defects were partially covered by cartilaginous material extending from the drill holes. Chondrocytes were in the base of the drill holes and mitotic activity in these cells was confirmed by tritiated thymidine uptake. Safranin-O staining was intense in the repair tissue but disappeared between eight and twelve months. The hyaline morphology of chondrocytes deep within the drill holes was maintained from eight to twelve months, but the collagen orientation remained random. Additionally, the hyaline-like morphology decreased toward the surface, becoming a mixture of fibrocartilage and fibrous connective tissue. The defects were completely resurfaced at twelve months, but the surface repair tissue became dense fibrous connective tissue. Mitchell and Shepard concluded that although the character of the repair tissue became dense and fibrous superficially over time, the presence of this repair tissue was superior to control lesions without drilling which had no repair tissue in the same postoperative period.

A variation of the "Pridie Procedure" referred to as "spongialization" was developed by Ficat et al in 1975 for treatment of chondromalacia of the patella.[117] The principle of using the subchondral bone to provide repair tissue was maintained, but Ficat resected not only diseased cartilage but also the entire underlying subchondral bone plate rather than perforating the plate with multiple small drill holes. The rationale was that frequently the subchondral bone itself was abnormal in this disease as evidenced by its poor consistency and relative ischemia. Another postulated benefit was protection of repair tissue by creation of a depression which would not be subjected to weight-bearing during early regeneration. Removal of subchondral bone was also theorized to eliminate a source of pain during the repair phase since subchondral bone is well-innervated. Some histologic specimens were available although the study was primarily clinical case follow-up. The operative area healed with tissue from the marrow spaces which was hyaline cartilage deeply and more fibrous tissue superficially. Clinically 79% of patients reported good to excellent results and postoperative pain was generally reported to be minimal. The procedure was proposed for treatment of severe and localized chondromalacia of the patella although thorough histologic assessment of the procedure was not available.

Attempts to characterize the repair tissue in subchondral drill holes using biochemical analysis were reported by several investigators in the 1980's. Cheung et al (1980) made 6-mm diameter defects in the femoral condyles of rabbits.[93] Histologic and biochemical analysis showed that up to 10 weeks after surgery the predominant collagen in repair tissue was Type II, the normal collagen of hyaline articular cartilage. Furukawa et al (1980) more precisely characterized collagen types in relation to postoperative time in a subchondral drilling model in rabbits.[118] Type I collagen predominated between three to six weeks, but Type II collagen was recognized after three to four weeks and became predominant six to eight weeks after surgery. Type II collagen remained predominant for the one-year period of study, but significant quantities of Type I collagen persisted throughout the study. Glycosaminoglycan (GAG) content decreased steadily until it was less than control cartilage at six months. The surface of many defects became fibrillated after six months, concurrent with the decrease in GAG content. Furukawa concluded that the significant amount of Type I collagen which persists at one year suggests that normal hyaline cartilage never fully develops and may lead to biomechanical failure of the repair tissue. Biomechanical failure and the resulting loss of proteoglycans eventually results in the fibrous nature of the repair tissue superficially rather than a specific change in collagen production by the chondrocytes. The study did not examine the ultrastructure or morphologic characteristics of repair tissue after eight weeks to assess if the late changes were the result of degeneration, a specific change in cell type, or a combination of both factors.

Vachon et al (1986) evaluated subchondral bone drilling as an adjunctive measure to curettage in surgical treatment of full-thickness cartilage defects in the horse.[119] Defects one centimeter in diameter were created down to bleeding subchondral bone in the radial facet of each third carpal bone in each horse. One defect was treated with five 1-mm diameter drill holes to a depth of 10 mm to assure penetration of the underlying cancellous bone; the defect in the contralateral third carpal bone was not drilled and served as the control defect. Routine synovial fluid analyses at one and three weeks postoperatively were not significantly different between drilled and control defects. Four horses had osteophytes located on the distal aspect of the radial carpal bone or the proximal third carpal bone at the termination of the study (21 weeks) and no synovial adhesions were present. As Grant observed, two types of repair tissue were identified grossly.[113] Vachon postulated that the thickness of the repair tissue rather than histologic differences produced the different gross appearances. The surface area covered by repair tissue and the thickness of repair tissue were significantly greater in drilled defects. Histologically, the tissue filling the drill holes and covering the drilled defects was fibrocartilage; no hyaline cartilage was observed. The fibrocartilage assumed a more fibrous character away from the drill holes. Staining for proteoglycans was intense in the drill holes and decreased away from the drill holes. The control defects were resurfaced with fibrous connective tissue. The repair tissue in drilled carpal bones was well-attached to the defect surface but attachment was poor in five of the six control defects. The fibrocartilage repair induced by subchondral drilling was felt to be superior to the fibrous repair in the control defects, and the technique was recommended to improve healing of third carpal bone cartilage lesions.

The effect of subchondral bone drilling on repair of partial-thickness defects was examined by Shamis et al (1989) in the horse.[120] Partial-thickness defects one centimeter in diameter were created in the radial facet of the third carpal bone. One carpal defect was treated with subchondral drilling and the defect in the opposite limb served as the untreated control. Drilling was performed with a 1-mm drill bit to a depth of one centimeter to penetrate the underlying cancellous bone; five or eleven drill holes were created. Drilled defects had significantly greater surface area coverage by repair tissue than undrilled defects, and the number of drill holes did not significantly affect surface coverage. Fibrocartilage was located in the depths of the drill holes, in agreement with Vachon[119], while fibrous connective tissue filled the superficial aspects of the drill holes and the areas adjacent to the drill holes. The repair tissue was firmly attached to the drill holes and adjacent cartilage, but was not frequently attached to the base of the defect. This focal attachment of repair tissue was considered questionable for its ability to withstand the stress of athletic performance. Proteoglycans were demonstrated only in the fibrocartilage in the drill holes, and the surface deficiency of proteoglycans was postulated to make the repair susceptible to trauma. As shown in other studies, two types of repair tissue were seen grossly: dense white glistening tissue and dull light-gray tissue. Shamis characterized these tissues histologically as fibrocartilage. The dull gray tissue was thin, primarily fibrous tissue with very low proteoglycan content and was found in areas above mineralized cartilage. The dense white tissue was fibrocartilage with proteoglycans and was found in drill holes and adjacent to drill holes. This dense white repair tissue was considered biomechanically superior to the dull gray tissue because of higher proteoglycan content and was therefore considered an acceptable repair. The dull gray tissue was considered unacceptable repair.

Increasing the number of drill holes from five to eleven did not have a deleterious effect on the synovial fluid analysis and did not improve histologic results; however, an increased number of drill sites theoretically would improve attachment of repair tissue.

Lesions in Cartilage Opposing Articular Cartilage Defects in the Horse

The biochemical changes which occur in articular cartilage opposing both full- and partial-thickness lesions were characterized by Richardson and Clark (1990).[121] Lesions one centimeter in diameter were created on the proximal surface of the intermediate carpal bones, approximately 7-mm from the dorsal articular rim. Partial-thickness defects were created in one carpus, and the subchondral bone was penetrated in the opposite carpus. All samples were collected six weeks after surgery. The cartilage opposite partial-thickness defects was slightly raised above the level of surrounding cartilage. Glycosaminoglycan content of this tissue was not significantly different from the initial glycosaminoglycan content in samples taken during surgery. Cartilage opposite full-thickness defects was raised and roughened.

Glycosaminoglycan content was markedly decreased and synthetic activity as determined by radiolabelled sulfate uptake was increased in the cartilage opposite full-thickness defects. Glycosaminoglycan content and synthetic activity were not significantly affected by partial-thickness lesions. The "kissing lesions" produced by full-thickness defects were of greater severity than those produced by partial-thickness lesions at six weeks. Since the gross, histologic and biochemical changes were localized to the kissing lesions rather than the entire articular surface, altered biomechanical forces were postulated to be the primary factors in the development of degeneration of the opposing cartilage surface.

Presently, complete healing of full-thickness articular cartilage defects with normal articular hyaline cartilage is not documented clinically or experimentally. Most current surgical treatments of cartilage lesions are directed toward resurfacing of defects with a biomechanically acceptable tissue and minimizing factors which incite and perpetuate degenerative joint disease. Further work is needed to explore factors which promote normal hyaline repair tissue in articular cartilage defects.

MATERIALS and METHODS

Experimental Design

Six healthy horses ranging from two to eight years of age were included in the study. Each animal was examined clinically and radiographically and found to be free of carpal disease. Flexed lateral Xeroradiographs of each carpus were obtained and served as the preoperative baseline study. Skeletal maturity was indicated by closure of the distal radial physis of both limbs in all horses. Horses were randomly assigned to one of two experimental groups which were based upon the postoperative duration of study. The duration of study was four months (experimental group 1, n=3) or six months (experimental group 2, n=3).

Tetanus toxoid was administered, and an anthelmintic was administered to all horses prior to surgery. Horses were fed a mixture of grass and legume hay ad libitum during the period of stall confinement and paddock exercise.

Surgical Procedure

Preoperative sedation was obtained by administration of xylazine (0.22 mg/kg, IV). Anesthesia was induced with guaifenesin (5% solution IV to effect) and ketamine (2.2 mg/kg, IV) and maintained with halothane and oxygen (inhalation to effect). The horse was positioned in dorsal recumbency on a warm waterbed, and the forelimbs were suspended in a moderately flexed position for the surgical procedure. The dorsal aspect of each carpus was routinely prepared for aseptic arthroscopic surgery of the intercarpal joint.

An arthroscopic portal was created in the intercarpal joint by stab incision one centimeter lateral to the common digital extensor tendon. The instrument portal was made one centimeter medial to the extensor carpi radialis tendon. A motorized arthroscopic burr was used to create a 1-cm diameter defect in the proximal dorsal articular surface of the radial facet of the third carpal bone. The defect penetrated the calcified cartilage to expose bleeding subchondral bone in one carpus (superficial defect) and was extended through the subchondral bone plate (6-mm to 8-mm depth) in the opposite carpus such that the entire subchondral plate was removed (deep defect). The removal of the subchondral bone plate was performed according to random block design (in the right carpi of 3 horses and in the left carpi of three horses). The walls of the defects were made vertical with a bone curette. The joints were liberally lavaged with sterile lactated Ringer's solution. The skin of each stab incision was apposed with 2-0 monofilament nylon in a vertical mattress pattern. A sterile pressure bandage was applied to each carpus.

Postoperative Care and Evaluation

Phenylbutazone (4.4 mg/kg, PO) was administered beginning two hours prior to surgery and continued once daily for five days. Procaine penicillin G (22,000 IU/kg, IM) was administered two hours prior to surgery and continued once daily for five days. Skin sutures were removed on the tenth postoperative day. Bandages were maintained and changed as needed for 21 days after surgery. Stall confinement was provided for two weeks followed by small paddock exercise for three weeks. Pasture turnout began in the sixth postoperative week. Horses were observed daily for general well-being while turned out.

Clinical examination including evaluation of lameness was performed daily for the first 14 days after surgery, every other day for the next three weeks and then at four week intervals until the termination of the study. Horses were examined to detect soft tissue swelling, joint effusion and resistance to flexion of each carpus. Lameness was evaluated at the walk and trot prior to euthanasia, although horses were observed daily for lameness during the period of pasture exercise.

Flexed lateral Xeroradiographs of each carpus were taken prior to surgery and at four-week intervals after surgery until termination of the study. Xeroradiographs were examined for soft tissue swelling, periosteal proliferation, location and degree of osteophyte formation and response of the subchondral bone of the third carpal and radial carpal bones.

Arthrocentesis of each intercarpal joint was performed immediately prior to euthanasia. The color and transparency of the fluid was recorded. A refractometer was utilized to measure total solids. White blood cell counts were performed on a standard hemocytometer following dilution and red cell lysis. The quality of mucin clot formation was evaluated by adding 2 ml of 2% acetic acid to 0.5 ml of synovial fluid and observing the precipitate. Air-dried smears of each sample were stained with Wright's stain and evaluated microscopically for cell type and morphology.

Collection of Specimens

Group 1 horses were humanely euthanized at four months and group 2 horses at six months after surgery. Each carpus was removed, and the intercarpal joints were dissected and photographed. The joints were examined grossly for the presence and location of osteophytes and synovial adhesions, thickening and proliferation of the joint capsule and synovial membrane and changes in the opposing surface of the radial carpal bone. The surgical defects were evaluated subjectively for surface area coverage, color, thickness and consistency of repair tissue.

The intermediate and third carpal bones of each carpus were dissected for microscopic examination. Specimens were fixed in 10% buffered neutral formalin (BNF) for 24 hours, then dehydrated in 70% ethanol. Decalcification of osseous tissue was performed by resin ion exchange with 10% formic acid under continuous agitation for 2 weeks. The specimens were then embedded in paraffin, sectioned and stained with hematoxylin and eosin and safranin-O-fast green and were examined by light microscopy. The origin and type or types of tissue in the repair, extent of attachment of repair tissue to underlying and adjacent tissues, changes in subchondral bone and cartilage adjacent to the defect and staining characteristics of repair tissue were evaluated.

Synovial membrane from each joint was collected from a location lateral and distant from the incision site. Synovial membrane specimens were fixed in 10% BNF, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Sections were examined with light microscopy.

Statistical analysis was performed on the numerical data from the synovial fluid analyses. Analysis utilized Wilcoxon's signed rank test for within group comparisons and with Wilcoxon's rank sum test for comparisons between the experimental groups. Differences were considered significant when $p<0.05$.

RESULTS

Clinical Examination

Moderate soft tissue swelling and intercarpal joint effusion was palpated over the dorsal aspect of all carpi for the initial 5 to 7 postoperative days. Minimal soft tissue swelling persisted over the intercarpal joints for 6 to 8 weeks after surgery. There was a slight decrease in carpal range of motion in the initial postoperative period which disappeared during the first month. Lameness at the walk was not observed early or during the period of paddock or pasture exercise. All horses were sound at the walk and trot prior to euthanasia.

Xeroradiography

Moderate soft tissue swelling was apparent xeroradiographically over all intercarpal joints until two months after surgery. Soft tissue density was minimal at three months and approached normal by the fourth postoperative month. Capsular calcification was evident at the intercarpal joint capsule attachment to the radial or third carpal bone or at both locations (Figure 1). The capsular calcification was usually mild at the first month after surgery, increased over postoperative months two and three, and was unchanged or slightly decreased by the fourth month after surgery. In all horses, the capsular calcification appeared more pronounced in the joint with the subchondral bone plate removed than in those with superficial defects.

Marginal osteophytes were observed in four of the six joints in group 1. Two of these joints had the subchondral bone plate removed. Osteophytes were located at the distal articular margin of the radial carpal bone or the proximal articular margin of the third carpal bone (Figure 2). The osteophytes in joints with the deep defects were larger than those in joints with the superficial defects.

Osteophytes were seen in four of the six joints examined at six months, and all were located on the distal articular margin of the radial carpal bone. Three of the affected joints contained deep defects.

The response of the distal radial carpal subchondral bone was visible as focal lysis opposite the defect one month after surgery in most cases (Figure 1). Normal contour of this area was not reestablished during the study period. One horse in group 1 developed bilateral osteochondral fragments from the distal radial carpal bone margins which were discovered one month after surgery. The fragment in the carpus with the deep defect was large and displaced (Figure 2) while the opposite fragment was small and non-displaced. Another group 1 horse had a non-displaced osteochondral fragment from the distal articular margin of the radial carpal bone in the joint with the superficial defect.

Synovial Fluid Analysis

The color and transparency of synovial fluid obtained from each intercarpal joint prior to euthanasia was straw-colored and clear (9/12 joints) except when contamination with peripheral blood occurred during arthrocentesis, producing an amber colored, hazy fluid (3/12 joints). The mucin precipitate quality was subjectively graded as excellent in all samples.

The total protein concentration in the 4-month group (Table 1) ranged from less than 2.5 gm/dl (lowest value on the refractometer) to 2.6 gm/dl in the joints with deep defects. In the 6-month group (Table 2), the total protein ranged from less than 2.5 gm/dl to 2.5 gm/dl in joints with the deeper defects. All joints with superficial defects had total protein values less than 2.5 gm/dl in both experimental groups. No significant difference in synovial fluid total protein was measured between superficial and deep lesions within either experimental group or between groups 1 and 2.

In group 1, white blood cell counts ranged from 0 to 440/mm^3 in joints with superficial defects (Table 1). In joints with deep defects the WBC counts were 110 to 1540/mm^3. The cells were non-degenerative mononuclear cells, except for rare polymorphonuclear cells in two samples with peripheral blood contamination. In group 2, white blood cell counts in joints with superficial defects ranged from 0 to 110/mm^3 (Table 2). Joints with deep defects had WBC counts from 40 to 220/mm^3. White blood cell morphology was similar to that of experimental group 1. White blood cell counts were not significantly different between joints with superficial and deep lesions. No significant differences existed in WBC count between the experimental groups.

Gross Necropsy Observations

The surgical incisions were completely healed in all joints. There was no gross evidence of soft tissue inflammation in any specimen. The joint capsule of all operated joints was mildly thickened when compared to the joint capsule of unoperated carpal joints. The appearance of the joint capsule and synovial membrane was similar between group 1 and group 2 specimens regardless of lesion depth. Synovial adhesions were not present in any joint.

The surgically-induced defects were easily identified in all third carpal bones. The following two types of repair tissue were identified grossly: a cream-colored, dense tissue and a dull grey, thin tissue. The depth of the surgical lesion could be identified in each specimen by the relative quantities of each type of repair tissue.

In superficial defects of the 4-month group, repair tissue did not completely resurface the defects in that it did not extend to the margins of the defects and was not uniform in thickness. The repair tissue was primarily thin and grey although rare islands of the thicker cream-white tissue were seen. Surface coverage was focal and incomplete.

Deep defects in the four-month group were primarily covered by thick, cream-colored tissue that frequently extended completely to the margins although in no specimen was the defect completely covered. The repair tissue was more uniform in thickness than was seen in the superficial defects.

The gross appearance of six-month defects was similar to those after four months. Superficial defects had focal areas of coverage by both types of repair tissue although surface area coverage was slightly more complete than that of four-month specimens. The surface coverage by cream-colored tissue in deep defects was very nearly complete, with an infrequent margin left uncovered. The repair tissue in the deeper defects closely approximated the surface level of adjacent hyaline cartilage.

The distal surface of the intermediate carpal bones was grossly normal in all joints. Other articular surfaces not in contact with the surgical defects appeared grossly normal in all specimens.

On all radial carpal bones, raised, circular areas corresponding to the size of the surgical defects were present on the distal surfaces opposing the third carpal defects. The distal radial carpal lesions were dull white in contrast to the appearance of distant articular surfaces which were glistening grayish-white. The presence and location of osteophytes corresponded to the xeroradiographs.

Histopathology

The origin of repair tissue in the third carpal bone defects was clearly the subchondral marrow spaces when they were exposed (Figure 3). Fibrous tissue appeared to emanate from the margins of some defects. In areas with remaining calcified cartilage, the subchondral bone did not appear to contribute to the repair. These areas were covered partially or completely by fibrous tissue which appeared to originate from superficial fibrous layer of repair tissue and was occasionally continuous with the superficial layer of adjacent hyaline cartilage. This fibrous connective tissue was not attached to the calcified cartilage at any site (Figure 4).

The types of repair tissue varied according to lesion depth. In superficial defects, fibrocartilage formed a layer just above the subchondral bone and fibrous connective tissue formed a superficial layer above the fibrocartilage (Figure 5). The fibrocartilage was not oriented in any one direction; however, the collagen fibers in the fibrous tissue were aligned parallel to the articular surface. The marrow spaces in the subchondral bone contained either fibrocartilage or fibrous connective tissue or mixtures of both tissue types. Histologic characteristics of the repair tissue did not vary between 4- and 6-month groups.

In the deep defects of the 4-month group, the depths of the defects contained a layer of hyaline-like cartilage with aggregated chondrocytes. Above the hyaline-like tissue was a layer of fibrocartilage with an overlying layer of fibrous connective tissue with collagen fibers oriented parallel to the surface (Figure 6). Six-month specimens were similar except that the deep layer of repair contained a mixture of hyaline-like cartilage and fibrocartilage.

The extent of attachment of repair tissue to the underlying subchondral tissue was good in all specimens except where calcified cartilage remained. Large clefts between the repair tissue and mineralized cartilage were observed (Figure 4).

Attachment of repair tissue to the cartilage adjacent to the defect was not frequently observed in either experimental group. Perpendicular clefts were frequently observed between the adjacent cartilage and tissue filling the surgical defects. When attachment was present it occurred through a narrow acellular zone of fibrous repair tissue (Figure 7). The depth of the surgical defect did not appear to influence the attachment of repair tissue to adjacent cartilage.

In the four-month specimens the superficial fibrous tissue was oriented parallel to the articular surface. Deeper tissue layers had no specific orientation of collagen fibers (Figure 5). In each of the six-month defects, the collagen of the superficial fibrous layer was aligned parallel to the surface, and two of the deeper defects had perpendicular alignment of collagen fibers in the deepest layers of the repair tissue which was not uniform and tended to occur at random locations across the base of the defects (Figure 8). Normal articular cartilage zonation was not reestablished in any specimen.

The subchondral bone plate was not reestablished in any defect where it had been removed. The subchondral bone plate in joints where it was exposed had an irregular contour and was not level with the subchondral bone adjacent to the defects. Fragments of necrotic woven bone were occasionally seen in the deep layers of hyaline cartilage repair tissue (Figure 3). The bone beneath the defects was sclerotic in all specimens, but the cancellous bone beneath adjacent normal articular cartilage did not appear sclerotic.

The cartilage adjacent to the defects had a similar histologic appearance regardless of lesion depth or experimental group. Cell columns of the adjacent cartilage tilted toward the defect, producing mild lipping over the superficial margins of the defect. Chondrone formation was prominent in the areas of lipping and was less prominent away from the defect. Where attachment to fibrous repair tissue had occurred, lipping and chondrone formation were still observed adjacent to the acellular fibrous zone of attachment (Figure 7). Staining with safranin-O was pale in the cartilage adjacent to the lesion but was normal away from the defects.

Safranin-O-fast green staining characteristics were similar in all third carpal bone defects from both experimental groups. The superficial fibrous connective tissue did not retain safranin-O. Fibrocartilage stained lightly in pericellular regions and hyaline-like cartilage stained moderately intense to intensely with safranin-O.

Histologic appearance of the intermediate carpal bones was similar in all joints. All specimens had surface fraying of the tangential zone but the remaining articular cartilage structure was preserved. Staining of the cartilage with safranin-O-fast green was normal on all intermediate carpal bones.

Synovial membrane had a similar histologic appearance in all joints. Mild villous hyperplasia was present with occasional foci of organized fibrin over the villus tips. A mild increase in mononuclear inflammatory cells in the subintima was seen in one specimen in experimental group 2; a superficial defect was created in this joint.

DISCUSSION

The radial facet of the third carpal bone was selected for creation of experimental defects because of the high incidence of chondral and osteochondral injury to this area.[122,123] Additionally, results of athletic performance following surgical treatment of chip fractures at this location are less successful than other locations in the carpus.[123] The articular surfaces of the third carpal bone are subjected to high impact forces, therefore biomechanical effects on repair tissue are prominent at this location.[119] The subchondral bone of the proximal third carpal bone is thick in comparison with other carpal bones and may affect the availability of a source of repair tissue.[123] Finally, since this site has been used by other investigators, information is available on repair of experimental lesions in the third carpal bone.[119]

The one-centimeter diameter of experimental defects was selected based on earlier work which reported that defects 9-mm and larger in diameter do not heal completely.[94] The defects through the subchondral bone plate were created to a depth of 6 to 8-mm to penetrate the articular cartilage (2 to 3-mm thick) and subchondral bone (4-mm thick) and adequately expose the cancellous marrow spaces.

The postoperative exercise regime was intended to simulate the typical postoperative recovery of clinical cases treated with carpal arthroscopy. Group 1 horses were euthanized four months after surgery, which is the recommended recuperative period following surgical treatment of clinical cases with osteochondral defects of the third carpal bone.[113] Grant (1975) showed that after four months healing does not appear to progress.[113] Furthermore, four months has been shown to be adequate time for repair tissue in the third carpal bone to undergo metaplastic change to fibrocartilage.[6] Imperfect hyaline cartilage has been shown to fill third carpal bone defects by six months postoperatively,[6] therefore group 2 horses were euthanized at six months to examine further changes in the type of repair tissue filling the defects.

Clinical examinations after surgery indicated a routine post-arthroscopy recovery. The intercarpal joint effusion, soft tissue swelling and decreased range of motion are common findings following arthroscopic surgery. It was not possible to distinguish lesion depth by clinical examination at any time following surgery, indicating that similar periarticular environments existed independent of lesion depth. Evidence of chronic degenerative joint disease was not detectable by physical examination in any joint, although some did have radiographic changes suggestive of degenerative disease. It is possible that the four- and six-month periods of study were insufficient for clinical signs of degenerative joint disease to develop.

Bone proliferation at the joint capsule attachment (capsular calcification) is a radiographic change associated with degenerative joint disease and is believed to result from tearing or strain on the joint capsule.[123] Capsular calcification occurred in all intercarpal joints, indicating that some degree of degenerative joint disease was present in all joints, presumably in response to the surgical defects rather than traumatically-induced. The proliferative response was more severe in response to the deep defects, suggesting that degenerative joint disease was more pronounced in these joints. It appears that capsular calcification can accompany degenerative joint disease without specific trauma to induce its formation.

Marginal osteophyte formation is a nonspecific change associated with degenerative joint disease although the pathogenesis is not yet established.[123] The incidence of marginal osteophytes is not well-correlated to the severity of articular cartilage damage.[124] In the present experimental model osteophytes were observed xeroradiographically as early as eight weeks. This is earlier than previous reports of 32 weeks[6] and 21 weeks.[119] Vachon performed radiographic examination prior to surgery and at 21 weeks after surgery, therefore the initial time of osteophyte formation was not specifically identified.[119]

The location of osteophytes in areas adjacent to the surgically-induced defects is in agreement with other studies.[6,119] Riddle suggested that direct mechanical stimulation from the surgical defects produced the marginal changes in these locations since areas distant from the defects did not form osteophytes.[6]

Synovial fluid analysis confirmed that chronic active synovitis was not established in any joint. This result correlated with the clinical absence of intercarpal joint effusion and absence of lameness prior to euthanasia. The synovial fluid analysis does not establish the presence or absence of chronic degenerative joint disease since cell counts are dependent upon the amount of synovitis at the time of arthrocentesis and are highly variable.[123] The depth of the induced surgical defects did not incite different degrees of chronic joint inflammation as demonstrated by synovial fluid analysis and synovial membrane histology.

Gross examination of intercarpal joints indicated similar effects on soft tissue and articular surfaces away from the radial facet of the third carpal bone regardless of lesion depth. The lack of grossly evident soft tissue inflammation correlated with the synovial fluid analyses.

The distal articular surface of intermediate carpal bones was grossly normal, corresponding to the histologic findings of minimal surface fraying and normal safranin-O-fast green staining.

Synovial adhesions to the surgical lesions were not seen in the present study. Other investigators have observed the presence of fibrous connective tissue or fibrocartilage in areas contacted by synovial adhesions.[6,113] Perichondrial pannus on the dorsal aspect of the third carpal bone has been associated with active bone resorption beneath the pannus tissue via osteoclasts originating in the reactive perichondrium.[114] Perichondrial pannus was not seen in this study.

All radial carpal bones had opposing "kissing lesions" on their distal articular surface which corresponded to the shape and diameter of the surgical defects. The depth of the surgical defect did not alter the appearance of the kissing lesion, although a study of lesions opposing partial- and full-thickness proximal intermediate carpal lesions found that full-thickness defects produced more severe kissing lesions.[121] Local mechanical effects leading to loss of proteoglycans are proposed to be the primary factor in the development of kissing lesions, although the exact mechanism is not yet known.[121] Since both lesion depths in the present study removed physical contact from opposing cartilage, mechanical effects on the opposing surface were similar. The lytic appearance of subchondral bone on Xeroradiographic examination of the distal radial carpal bone may have represented local bone remodelling in the absence of normal mechanical compression from the opposing articular surface.

The gross identification of two types of repair tissue has been observed by other authors.[6,113,119,120] Other studies identified the dense white or cream-colored tissue as fibrocartilage or immature hyaline cartilage, and the thin grey tissue was primarily fibrous and often associated with synovial adhesions.[6,113] Vachon suggested that the gross appearance was related to tissue thickness rather than to the presence of fibrocartilage.[119] The present study found the cream-colored tissue to be a mixture of hyaline-like cartilage and fibrocartilage, while the grey tissue was a mixture of fibrocartilage and fibrous tissue. However, the repair tissue in the deep defects was thicker than that of the superficial defects, and the tissue thickness may have altered the gross impression of color. Shamis found that both types of tissue were fibrocartilage, varying in thickness and proteoglycan content.[120] The dull grey tissue was a thin layer of primarily fibrous tissue with low proteoglycan content, and the dense white tissue was fibrocartilage with higher proteoglycan content. The dull grey tissue covered areas where the mineralized cartilage layer was intact, and the dense white tissue was usually associated with repair of subchondral drill holes. The dense white tissue was considered acceptable repair tissue due to the higher proteoglycan content.[120] In the present study the gross appearance of repair tissue generally correlated with the depth of the surgical lesion, with removal of the subchondral bone plate giving the highest yield of thick, cream-colored repair tissue. Since this tissue is considered biomechanically superior due to the increased proteoglycan content, exposure of the subchondral marrow spaces would appear to enhance the quality of repair tissue based upon gross appearance of the tissue.

Surface area coverage by repair tissue was improved in the deeper defects and was better than reported results in other studies. Riddle reported 75% surface coverage of full-thickness defects in the third carpal bone at six months.[6] Another study of 4-mm full-thickness proximal third carpal bone lesions did not demonstrate complete filling at 17 or 67 weeks after surgery.[113] Weight-bearing, full-thickness defects of 15-mm diameter showed good repair at 2.5 months but at five months cleft formation between repair tissue and subchondral bone was common and eventually resulted in exposed subchondral bone.[114] Surface area coverage by repair tissue requires firm attachment to the defect base in order to withstand biomechanical forces from the opposing articular surface which are prominent on the proximal aspect of the third carpal bone.[119] Increasing lesion diameter is expected to increase these biomechanical influences.[114] Results of the present study indicate that surface coverage by repair tissue is enhanced by subchondral bone plate removal, and the surface coverage is maintained as long as six months after surgery. It is unclear whether this finding relates to improved attachment, improved source of tissue, improved biomechanical function of the tissue due to higher proteoglycan content or a combination of these factors.

All third carpal bone defects with exposure of subchondral bone (superficial defects) had fibrocartilage and fibrous connective tissue repair. Hyaline-like tissue was not identified in repair of these defects in this study, although other studies have confirmed the presence of varying amounts of hyaline-like tissue following exposure of the subchondral bone plate.[6,113] Metaplastic change of fibrocartilage to imperfect hyaline cartilage between four and six months as documented by Riddle[6] was not seen. This observation is in agreement with Grant who stated that the quality of repair following exposure of subchondral bone is probably maximal at four months.[113]

The present study demonstrated histologically that attachment of repair tissue to the defect base was adequate regardless of the depth of the defects. The exception to this finding was in areas where mineralized cartilage remained intact; no attachment was seen in these areas as has been previously reported.[114,120] It appears that once the mineralized cartilage is removed, attachment to the defect base will not be significantly affected by increasing lesion depth.

The absence of attachment or poor quality fibrous attachment of repair tissue to adjacent hyaline cartilage was frequently associated with cleft formation along the lesion margins. The defect margins probably represent an area susceptible to biomechanical trauma from the opposing articular surface with subsequent detachment and cleft formation.[114] Extensions of marginal clefts could theoretically undermine repair tissue along its base and result in exposed subchondral bone as seen in another study.[114] Results of the present study indicate that removal of the subchondral bone plate would not be expected to improve marginal or lesion base attachment of repair tissue over simply exposing the subchondral bone.

Marginal lipping of cartilage adjacent to the defects contributed minimally to surface coverage of some defects. Marginal lipping, also referred to as matrix flow, contributed minimally to lesion repair observed in other studies of full-thickness lesions greater than 8-mm diameter in the horse.[113,114] Matrix flow may be a more significant process in resolving smaller diameter (less than 5-mm) lesions.[91,114]

Chronic degenerative changes were established in all joints in this study. Superficial fraying of articular cartilage was present on all intermediate carpal bones indicating generalized synovitis.[123] The occurrence of capsular calcification in all joints is a radiographic change associated with degenerative joint disease. The majority of joints developed osteophytes in areas adjacent to the third carpal bone defects, therefore the osteophytes may have been a response to local mechanical factors.[6] Loss of intermittent compression may stimulate new bone growth locally in an attempt to reestablish contact and compression with the opposing surface. Osteophytes are considered to be a radiographic feature of degenerative joint disease although their presence correlates poorly to the severity of the disease.[124] Additionally, evidence of chronic mild inflammatory changes in the synovial membrane are compatible with degenerative joint disease.[125] It was not possible to associate the deeper defects with more severe degenerative joint disease than seen with superficial defects based on the available data. The creation of the deeper defects would theoretically be expected to incite more significant intra-articular inflammation due to liberation of bone particles which would incite synovitis or have a mechanically abrasive effect on other articular surfaces.[126] The clinical, radiographic and histologic findings indicate that degenerative joint disease affected all joints similarly, without regard to lesion depth.

Removal of the subchondral bone plate resulted in increased hyaline content of the repair tissue and increased surface coverage of the defects as compared to simply exposing the subchondral bone. Proteoglycan content of the deeper layers of repair tissue was also improved by removal of the bone plate. Attachment of repair tissue to adjacent cartilage and the defect base was not enhanced by creation of a deeper lesion. The long term fate of repair tissue was not determined in this study. Other studies have indicated that repair tissue will undergo degeneration over time and the hyaline cartilage-like nature of the repair tissue will decrease as a result.[113,118] If this degeneration does occur leading ultimately to fibrocartilage repair, the temporary appearance of hyaline tissue at the expense of inducing degenerative joint disease would not justify complete removal of the subchondral bone plate. Further work is needed to determine the long-term fate of repair tissue and the effects of degenerative joint disease following subchondral bone plate removal before this procedure can be advocated for clinical treatment for large diameter full-thickness cartilage defects.

Biomechanical studies are also needed to document the relative strengths of the fibrocartilage and hyaline-like cartilage that repair full-thickness cartilage defects. The biomechanical inferiority of fibrocartilage is suspected based upon decreased proteoglycan content relative to hyaline cartilage, but this has not been proven. The hyaline cartilage repair tissue is not normal in collagen content or architecture, and may not be superior to fibrocartilage when subjected to mechanical stresses. Fibrocartilage has been considered an acceptable repair tissue in articular defects.[105,127]

Two studies in the horse have reported results of penetration of the subchondral bone plate through 1-mm diameter subchondral bone drill holes to a depth of 1-cm.[119,120] These studies demonstrated fibrocartilage in the depths of the drill holes, while the present work clearly demonstrated hyaline-like cartilage in the depths of defects with complete removal of the subchondral bone plate. The depths of lesions in the former studies are comparable to the lesion depth in deep defects of the present study. Convery removed the subchondral bone plate in lesions of varying size in the equine femorotibial joint and observed a mixture of fibrous connective tissue and fibrocartilage at six months and hypercellular cartilage in the lesion depths at nine months.[94] Comparisons between femorotibial and intercarpal joint lesions may be affected by the different biomechanics of these joints. However, the present study found hyaline-like tissue in lesion depths at four months, differing from Convery's findings of nine months for appearance of hypercellular cartilage in lesions greater than 9-mm diameter.

The results of the present study indicate that exposure of the subchondral bone plate without exposure of cancellous marrow spaces and removal of the subchondral bone plate produce two distinctly different histologic results. Previous studies have not defined this difference in healing. The term "full-thickness" as applied to lesion depth and subsequent repair may therefore be inadequate for referring to these two depths of injury. It is suggested that the term "full-thickness" apply to the removal of all layers of articular cartilage to expose the subchondral bone without exposure of cancellous marrow spaces. When subchondral cancellous bone is exposed, this fact should be noted in addition to the term "full-thickness".

CONCLUSIONS

Complete removal of the subchondral bone plate in 1-cm diameter defects in the radial facet of the third carpal bone resulted in healing with a deep layer of hyaline-like cartilage, an intermediate layer of fibrocartilage and a superficial layer of fibrous connective tissue. Exposure of the subchondral bone without exposure of subchondral marrow cavities resulted in healing with a deep layer of fibrocartilage and a superficial fibrous connective tissue layer. Evidence of degenerative joint disease was observed in all joints. The amount of degenerative joint disease was not well-correlated with lesion depth.

Although hyaline cartilage content in repair tissue was improved by removal of the subchondral plate, in agreement with the experimental hypothesis, adverse degenerative effects produced in the joints preclude clinical use of the procedure as described for large articular lesions. Further work is needed to determine factors influencing differentiation of hyaline cartilage from pluripotential cells. Factors which could minimize the development of degenerative joint disease would also enhance the clinical efficacy of the procedure.

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FIGURE 1. Xeroradiograph, four month specimen, superficial defect. Capsular calcification is present on the dorsal aspect of the radial carpal bone (thick arrow). Subchondral lysis is present on the distal aspect of the radial carpal bone (thin arrow).

FIGURE 2. Xeroradiograph, four month specimen, deep defect. Marginal osteophyte formation is seen on the proximal articular margin of the third carpal bone (arrow). A large, displaced osteochondral fragment from the distal radial carpal bone and capsular calcification along both the radial carpal and third carpal bones are visible.

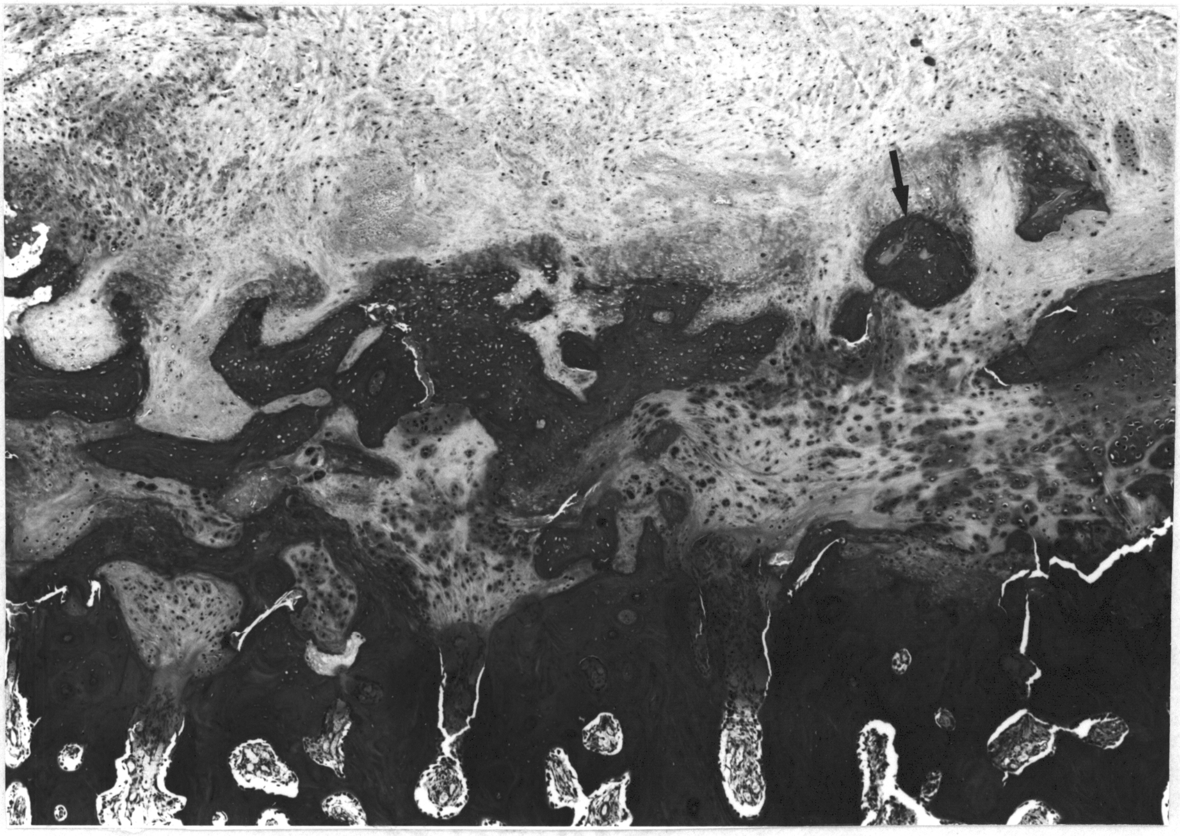

FIGURE 3. Four month specimen, deep defect. Hyaline-like cartilage originating in the subchondral marrow spaces fills the defect base. Fragments of necrotic bone with empty lacunae are seen above the deep layer of hyaline-like tissue (arrow). H&E, x 100.

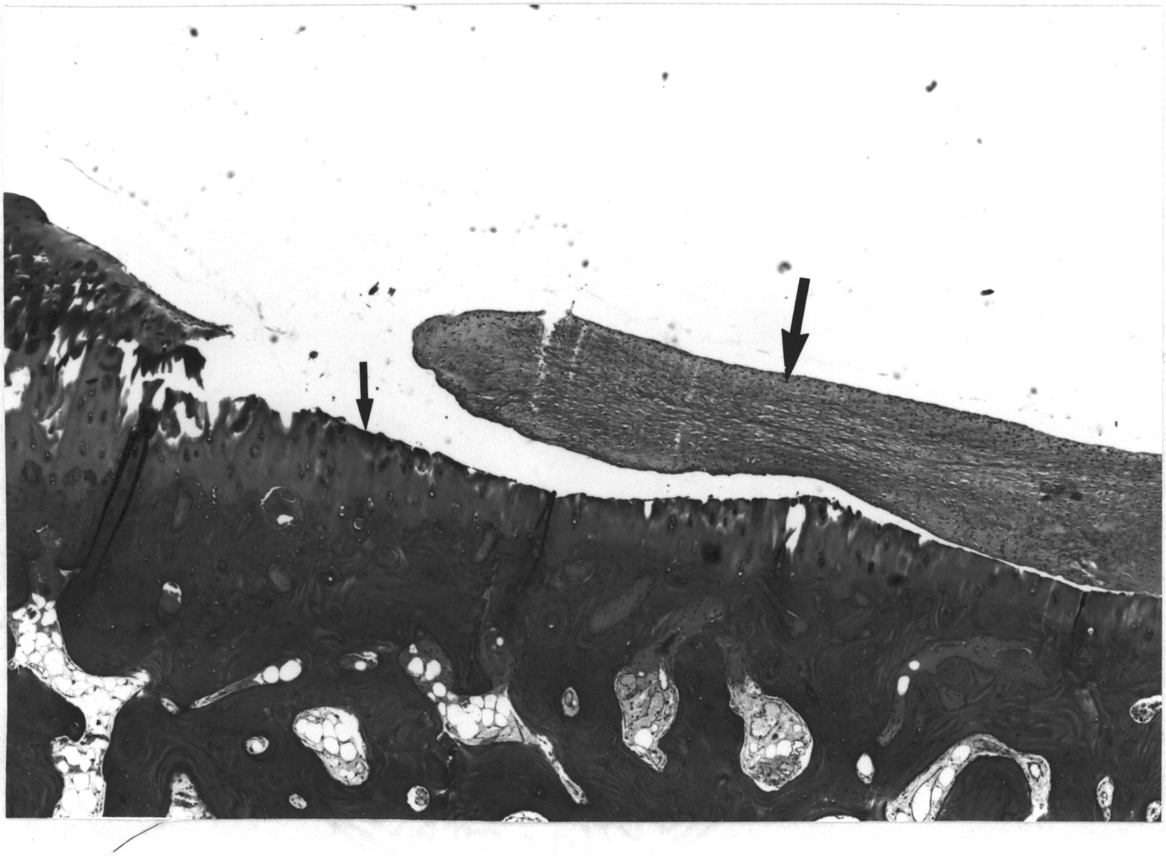

FIGURE 4. Four month specimen, superficial defect. Fibrous connective tissue (thick arrow) extends across an area of intact mineralized cartilage (thin arrow). The repair tissue is not attached to the mineralized cartilage and cleft formation is present. The subchondral marrow spaces are not contributing to repair in this area. H&E, x100.

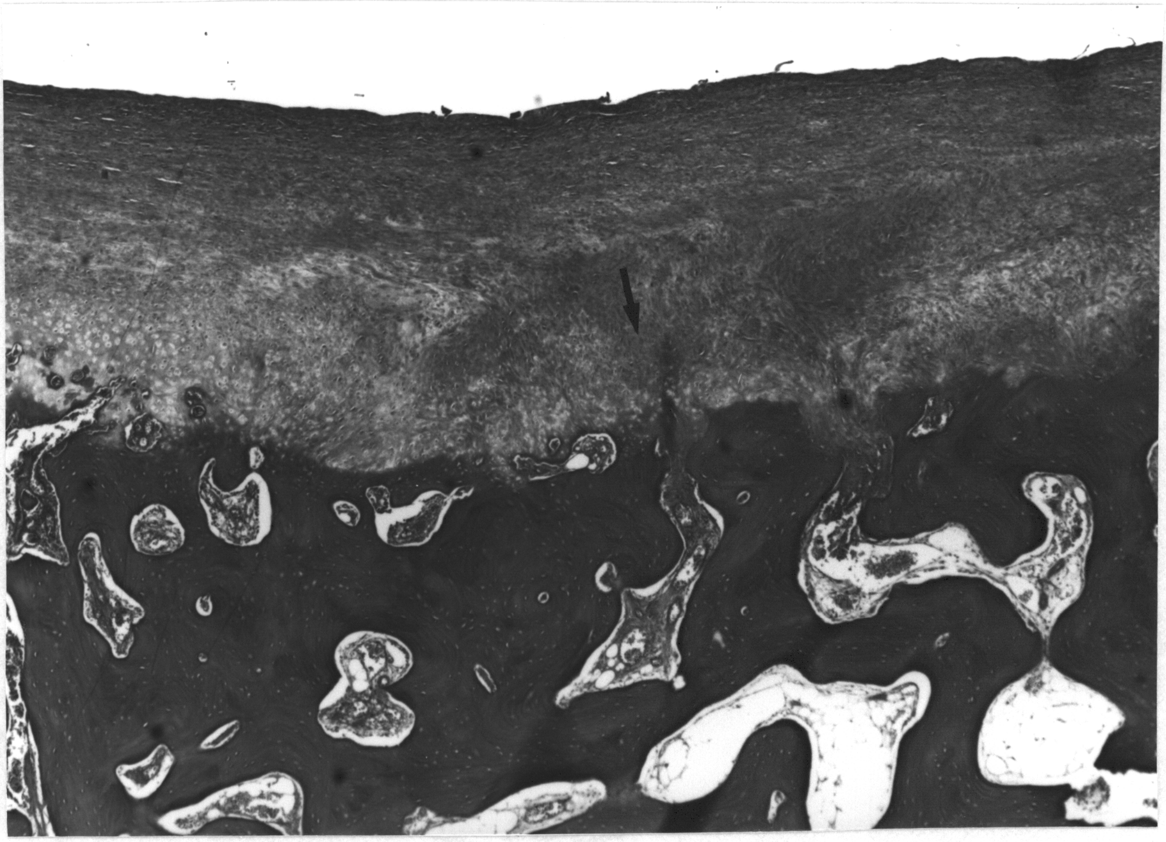

FIGURE 5. Six month specimen, superficial defect. Repair tissue consists of a deep layer of fibrocartilage and a superficial layer of horizontally-oriented fibrous connective tissue. Two subchondral marrow spaces contribute a small amount of hyaline-like tissue (arrow) to the deeper layer of repair. H&E, x100.

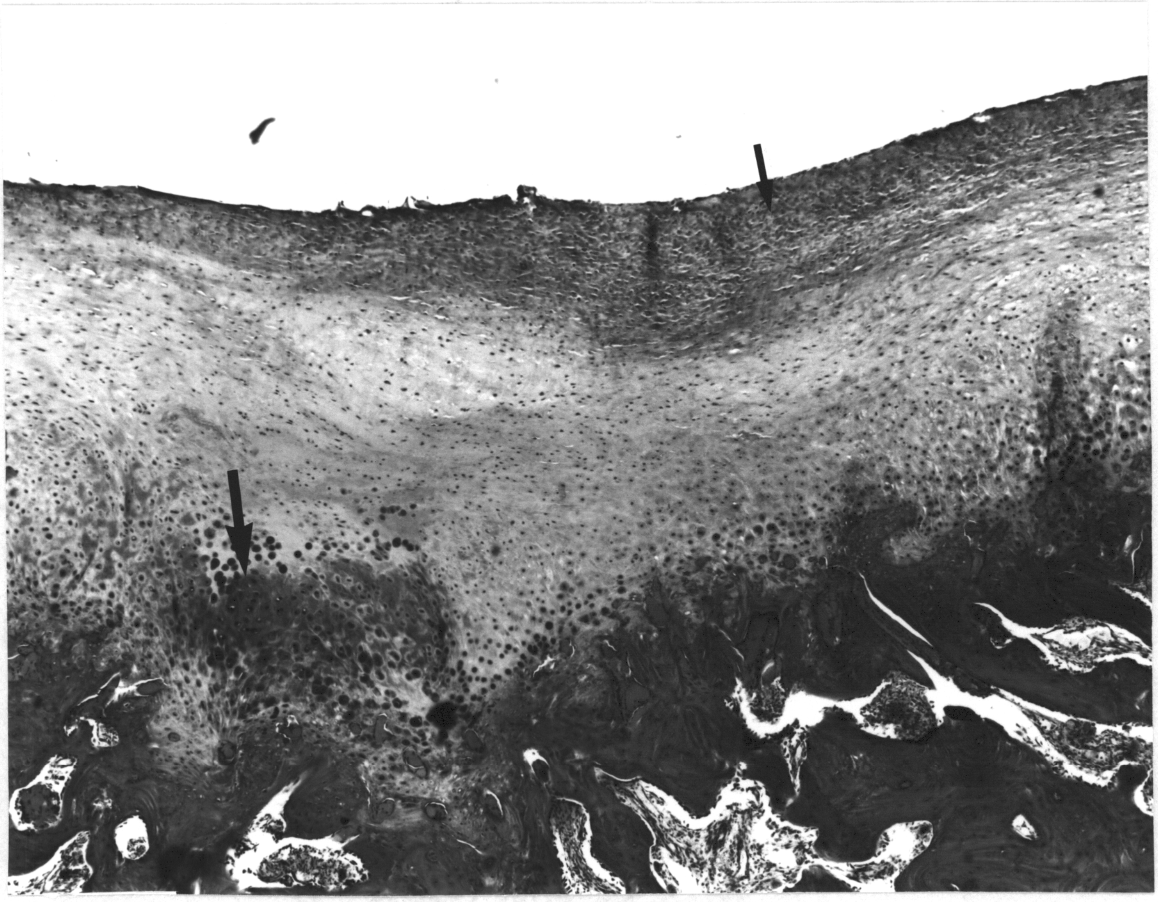

FIGURE 6. Four month specimen, deep defect. Hyaline-like tissue (thick arrow) originating in the marrow spaces forms the deep layer of repair. Fibrocartilage forms an intermediate layer and fibrous connective tissue (thin arrow) forms the superficial layer of repair. H&E, x100.

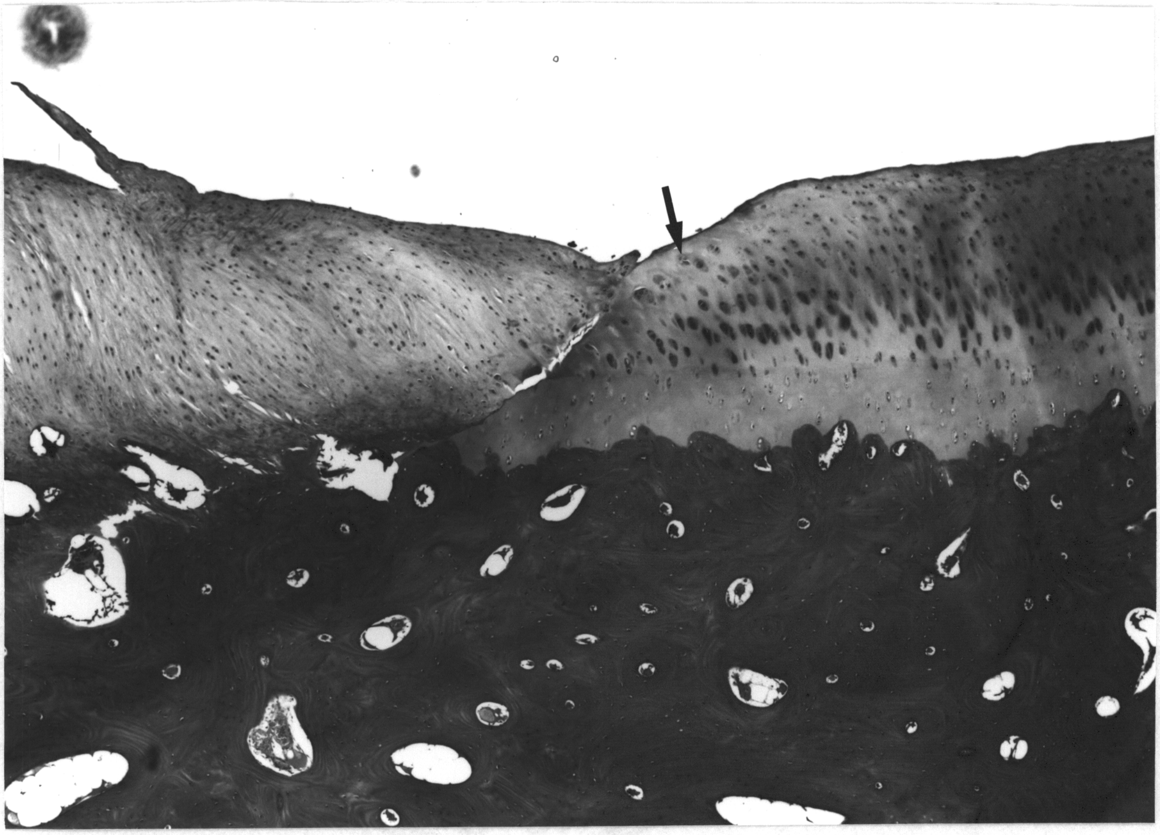

FIGURE 7. Six month specimen, deep defect. Narrow zone of attachment with cleft formation is observed between repair tissue on the left and adjacent hyaline cartilage on the right. Cell columns of the adjacent cartilage are tilted toward the defect. Chondrone formation (arrow) is present in the relatively acellular zone of adjacent cartilage. H&E, x100.

FIGURE 8. Six month specimen, deep defect. The deep layer of repair tissue shows a perpendicular alignment with respect to the joint surface. H&E, x100.

Table 1. Synovial Fluid Analysis, Experimental Group 1

Horse	Color/Transparency	Protein gm/dl	WBC /ml
1 superficial	yellow/ clear	<2.5	0
1 deep	amber/ hazy	2.6	110
2 superficial	amber/ hazy	<2.5	110
2 deep	yellow/ clear	<2.5	110
3 superficial	yellow/ clear	<2.5	440
3 deep	yellow/ clear	2.6	1540

Table 2. Synovial Fluid Analysis, Experimental Group

Horse	Color/Transparency	Protein gm/dl	WBC / ml
1 superficial	yellow/ clear	<2.5	110
1 deep	yellow/ clear	2.5	110
2 superficial	yellow/ clear	<2.5	0
2 deep	yellow/ clear	<2.5	40
3 superficial	yellow/ clear	<2.5	0
3 deep	amber/ hazy	<2.5	220

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