Yonsei Medical Journal
Vol. 31, No. 1, 1990

The Effect of Experimental Trypsin on the Regeneration of Hyaline Articular Cartilage

Chang-Dong Han and Ho-Jung Kang

There is evidence from other studies that some degree of cartilage healing may take place after the initiation of an inflammatory response. It is postulated that the induction of the platelet–cartilage interaction may eventuate in cartilage repair. The treatment of fresh articular cartilage with proteolytic enzymes rendered the tissue active as a platelet aggregant. During platelet aggregation a host of active substances are released which are known to play a role in the inflammatory response (Thompson 1975). This study was undertaken to evaluate the effects of trypsin on the surface injury of rabbit hyaline cartilage. The results were as follows: 1) Hyaline cell regeneration was observed only in the group treated with trypsin and blood; 2) Hyaline cartilage regeneration did not occur in the group treated with a single injection of trypsin or blood; 3) There was no significant damage to the healthy articular cartilage by the single injection of trypsin or blood, or both; and 4) Platelets do not adhere to cartilage and superficial damaged cartilage does not induce platelet aggregation.

Key Words: Trypsin, surface injury, articular cartilage

After the earliest record of Hunter (1743), all observers have demonstrated by gross, histological, and ultrastructural evaluation, the apparent inability of articular cartilage to produce surfficient new tissue to heal a superficial lacerative injury, or repair an ulcer on its surface. In such superficial injuries, confined to the substance of the cartilage of adult rabbits, the response has been shown to lack both the inflammatory and reapir components of the standard process as osberved in more vascular tissue.

In hyaline cartilage there are no local blood vessels to undergo vascular dilation, and the processes of transudation, exudation, and hematoma formation are absent. No fibrin is produced, so the fibrin clot that serves as the scaffolding for the ingrowth of repair tissue is absent.

In certain body tissue, the three phase response of repair is associated with the replication of the damaged tissue by the same tissue that was originally injured (bone-healing with bone callus; tendon-healing with tendon), rather than with a fibrous scar such as is seen in the healing of skin, liver, kidney, and so on (Zucker-Franklin and Rosenberg 1977).

Even injured adult articular–cartilage chondrocytes have the capacity to substantially increase their rate of matrix synthesis, and the possibility exists of chondrocyte participation in the repair of articular cartilage. The induction of platelet adhesion and aggregation on the articular cartilage in the living animal has been achieved. It is possible that factors released by platelets, which have been induced to adhere and aggregate on the surface of enzyme–modified cartilage, could promote the proliferation of chondrocytes and the synthesis of proteoglycans by these cells. The purpose of the present study was to evaluate the reparative response of superficial articular cartilage injury after a sequential intra-articular instillation of trypsin and autologous blood.

MATERIALS AND METHODS

The materials consisted of 30 full-grown rabbits with roentgenographically closed epiphyseal lines. The animals weighed 2900-3800g (mean 3100g). The rabbits were caged under normal conditions and given a normal diet. Under intravenous nembutal anesthesia, an arthrotomy was carried out in the right knee joint by a medial parapatellar incision.

A superficial longitudinal chondral defect of the

Received November 30, 1989
Accepted January 19, 1990
Department of Orthopaedic Surgery, Yonsei University College of Medicine Seoul, Korea
Address reprint requests to Dr. C-D Han, Department of Orthopaedic Surgery, Yonsei University College of Medicine, Seoul, Korea, C.P.O. Box 8044, 120-752

medial femoral condyle was made with a scalpel. The joint was then rinsed with a Ringer solution, followed by a precise fascia and skin suture.

There was no postoperative fixation of the knee joint. The rabbits were divided into four groups. At one week Group 1 (9 animals) was injected intraarticularly into the arthrotomized joint with 1.36mg trypsin in 1ml isotonic solvent; one week later, 1ml of its own this fresh blood was injected intraarticularly. Group 2 (7 animals) was given only trypsin, without a subsequent injection of blood. Group 3 (7 animals) was injected with 1ml of fresh autologous blood only two weeks after arthrotomy. Group 4 (7 animals) had no instillation at all after arthrotomy. Four weeks after arthrotomy, the animals were killed by an intravenous dose of nembutal.

Immediately after sacrifice, a full thickness sample of cartilage, measuring five by five millimeters, was removed. Each sample was divided into two blocks, and the blocks were fixed and embedded for light and electron microscopy, as will be described. For the light microscopy, the specimens were preserved in a 10 percent formalin solution. They were embedded in paraffin after decalcification.

The histologic sections were then stained with hematoxylin-eosin and alcian blue, and evaluated. The remaining samples were fixed in two percent glutaraldehyde in paraformaldehyde and 0.1M cacodylate, pH 7.4, for two hours; washed in a paraformaldehyde and 0.1M cacodylate solution; decalcified overnight in 4.13 percent EDTA, pH 7.4; and then fixed for an additional two hours in 1 percent osmium tetroxide. The samples were then rinsed in a buffer, dehydrated in a graded ethanol series, and embedded in Epon 812. The sections were stained with uranyl acetate and citrate, then examined with a Hitachi H-500 electron microscope.

RESULTS

The animals all survived the experimental period. There were no signs of any generalized toxic reactions to the administered trypsin. In six knee joints a histologic evaluation was impossible; three had infections, two had deep defects reaching the subchondral bone, and one did not allow for a clear histologic identification of the defect.

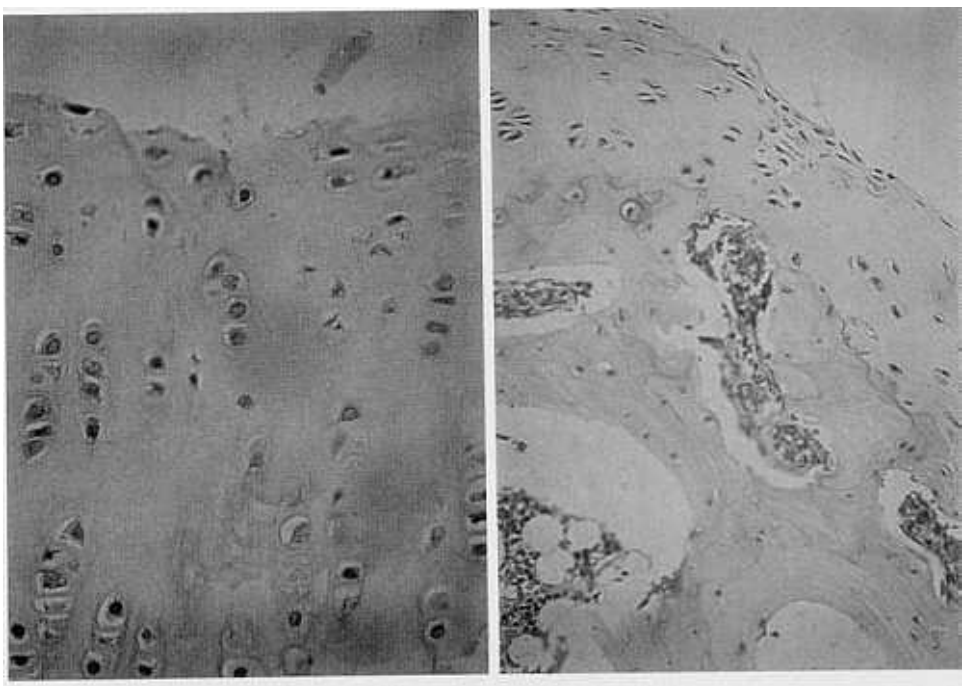

Fig. 1. A. Four weeks after sequential injection of trypsin and blood, the refill of the defect by chondroid cell tissue is shown (H and E × 200).

B. Four weeks after sequential injection of trypsin and blood, the refill of the defect by fibrous tissue is shown (H and E × 200).

Gross Findings

Except for the synovial thickening in the group treated with trypsin and blood, there were no distinct gross differences between the groups. The colour change of the articular cartilage was not observed in any of the groups.

Histological Findings

Group 1: The cartilage was seemingly normal except for a suspected fibrocartilage regeneration in three of the 9 joints. There was cloning of chondrocytes and hypercellularity at the regenerated area (Fig. 1). At the joint margins there was synovial proliferation and

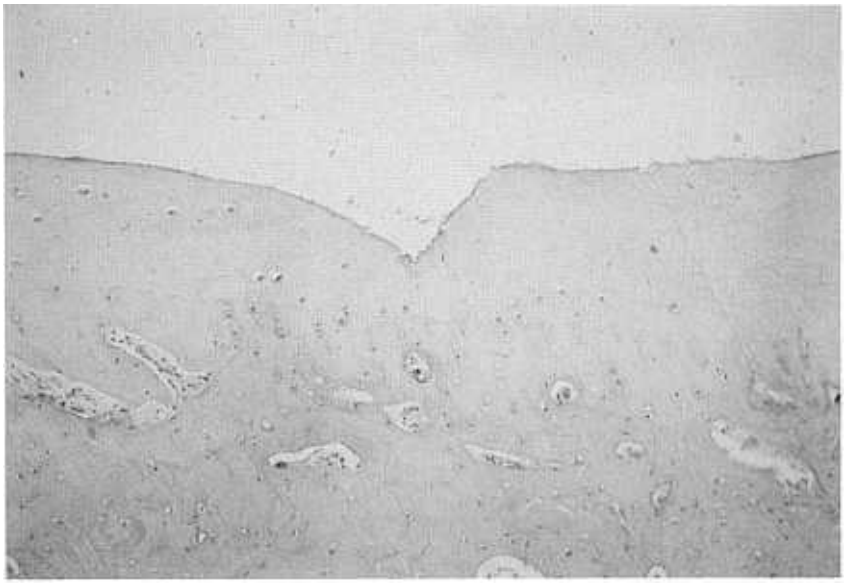

Fig. 2. Four weeks after trypsin injection only, no cartilage regeneration in the defected areas in shown (H and E × 200).

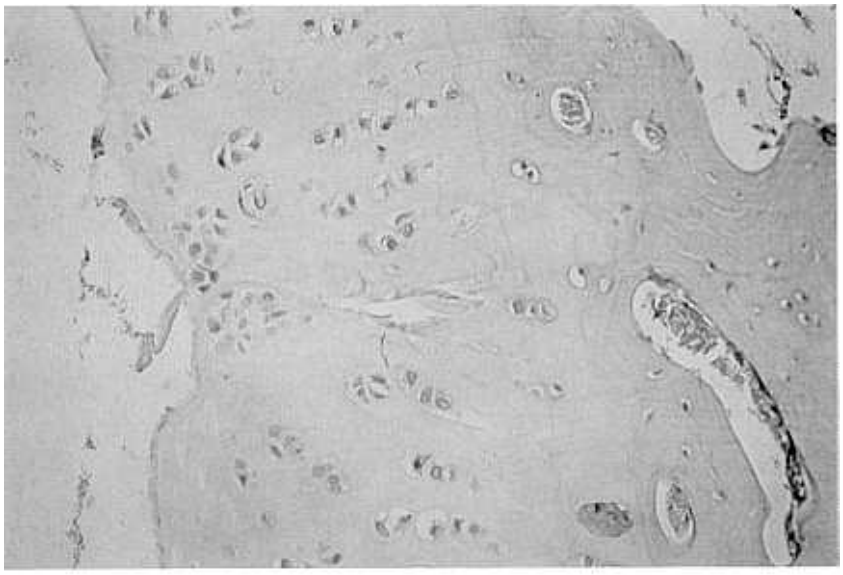

Fig. 3. Four weeks after blood injection only, no cartilage regeneration in the defected area is shown (H and E × 200).

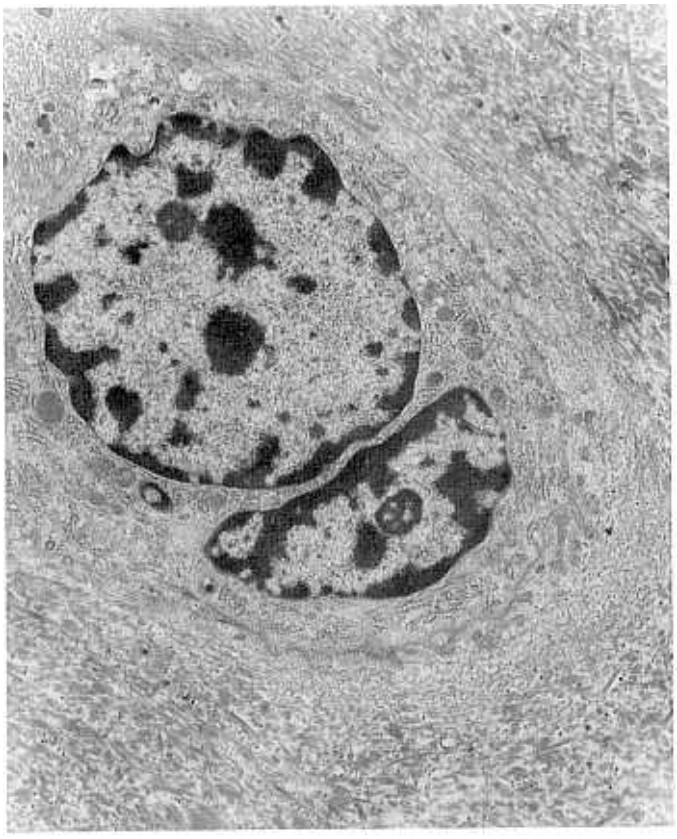

Fig. 4. In a four-week specimen after the injection of trypsin and blood, chondrocytes in the marginal area of the defected region show and abundance of intracytoplasmic fine filaments and glycogen lake (G). Mitosis of the chondrocytes is noted (×12,500).

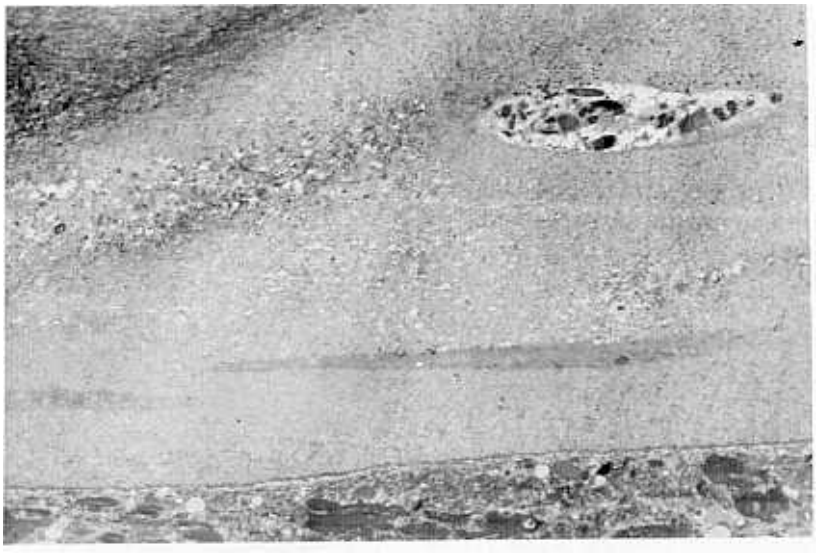

Fig. 5. Four weeks after trypsin injection only, superficial chondrocyte necrosis is seen. The surface-abutting joint space is encrusted with amorphous dense material and blood cells (×7,500).

thickening. The staining properties of the matrix were normal.

Group 2: The cartilage showed no histological signs of degeneration. Slight proliferative changes in the synovial membrane were seen at the margins. One of the 7 joints showed signs of degeneration with necrosis of the chondrocytes (Fig. 2).

Group 3: There was no reparative process in any of the superficial cartilage defects (Fig. 3). There was no change of staining properties of the matrix.

Group 4: None of the left control knee joints showed signs of degeneration. The staining properties of the matrix were normal.

Electron Microscopy Examination

The electron-microscopic findings confirmed the results of the histologic evaluation.

On electron microscopy of group 1, platelets had formed aggregates in association with the collagen fibrils. It was also shown that the chondrocytes began to divide, and that there was no histological degeneration of the chondrocytes (Fig. 4). On electron microscopy of groups 2 and 3, there was no aggregation between the platelets and the collagen fibrils. In group 2, two of the 7 knee joints showed signs of necrosis of the superficial chondrocytes (Fig. 5). The cartilage showed no signs of regeneration around the superficial defect area. In group 4, there were no signs of the regeneration of cartilage cells.

DISCUSSION

The articular cartilage lacks blood vessels, lymphatic vessels, and nerves; and once formed, it appears to remain unchanged unless it deteriorates. Thus, the physician caring for patients with joint diseases may believe that cartilage merits little interest beyond how to treat the symptoms of cartilage deterioration, and how best to replace the articular surface when it is damaged. Some of the controversy is based on the evident fact that cartilage responds to different forms of trauma in different ways. Full thickness defects (deep defects) violate subchondral bone healing by a proliferation of granulation tissue from the marrow spaces; however, partial thickness defects (superficial defects), which do not violate bone, very rarely heal or never heal (Fuller and Ghadially 1972).

When articular cartilage is damaged, the accompanying mitosis of the chondrocytes might be explained as an attempt to restore the cartilage. Mitosis of the chondrocytes is induced in adult joint cartilage in association with the development of osteoarthritic changes.

When degenerative changes in the articular cartilage develop in man or animals, the chondrocytes recover their ability to divide. It has also been shown that the chondrocytes begin to divide when there are no histological signs of degeneration in the cartilage. Local traumatization of the cartilage results in the mitosis of the chondrocytes, and also the absence of degenerative changes in the articular cartilage (Mankin 1982).

Degenerative joint disease can be induced in many different ways, i.e., mechanical, chemical, immunological, endocrinological and by infections. It is more difficult to explain why mitosis occurs when the degenerative changes are sparse or even absent. After the intra-articular injection of concentrated proteolytic enzyme solutions, extensive degenerative and necrotic changes of the joint cartilage appear. In the remaining cartilage, dividing chondrocytes can be seen.

Papain damages the proteoglycan molecules, resulting in the release of the glycosaminoglycans from the protein core. The occurrence of mitosis might be an attempt to repair the damaged cartilage (Farkas *et al.* 1975; Havdrup *et al.* 1982). If the theories of "chalones" are applied to this result, the findings can be explained. It is also possible that papain removes mitosis inhibiting molecules from the cell membrane. Perhaps these molecules could be the "chalones" described by Bullough and Laurence (1960). In a number of tissues, it has been found that mitotic activity is controlled by specific mitotic inhibitors, called chalones. Weiss and Kavanau (1957) proposed that a "negative feedback inhibitor" pre-existed in, or on, the surface of cells which specifically inhibited the mitosis of those cells until they were either displaced or destroyed. In the absence of these specific negative feedback inhibitors of mitosis, the cell would enter into the mitotic cycle.

This negative feedback inhibitor could be called a "chalone", from the Greek word meaning to brake or slow down. Its primary biological characteristics are i) total cell specificity, ii) lack of species-specificity, and iii) reversibility. The melanocyte chalone activity is destroyed by heating to>50°C, or by treatment with trypsin, chymotrypsin, or neuraminidase (Boldingh and Laurence 1968; Houck and Hennings 1973). When applied to joint cartilage, the chalone might be reduced in the joint cavity either by chemical (papain and trypsin), or mechanical (osteoarthritis and scarification) factors.

In our study, treatment of rabbit knee joints with intra-articular trypsin one week before the injection

of blood resulted in adhesion and aggregation of platelets on the surface of the lesions. This platelet-cartilage interaction plays an important role in cartilage repair. Superficial-thickness defects which do not involve the subchondral bone show significant reparative changes up to four weeks. We observed the complete filling of the superficial thickness defects with mature cartilage by four weeks. This provides infomation on the regenerative capacity of superficial damaged cartilage cells by intraarticular sequential injection of concentrated trypsin and blood. Trypsin splits the peptide bonds which follow one or the other of the two positively charged sidechains, i.e., lysine and arginine (Bartholomew *et al.* 1985). After an injection of trypsin, a greater number of dividing cells were seen when the solution containing 15,000 E/ml was used. Why peak mitosis occurs with a trypsin concentration of 15,000 E/ml is difficult to explain. The animals which have received more concentrated solutions of trypsin did not show corresponding changes. The appearance of dividing chondrocytes can be regarded as an effect of chalone reduction due to trypsin digestion (Havdrup 1979).

There is evidence from other studies that some degree of cartilage healing may take place after the initiation of an inflammatory response. It is postulated that the induction of a platelet-cartilage interaction may eventuate in the cartilage repair. But platelets did not adhere to articular cartilage, and that cartilage did not induce platelet aggregation in vivo or in vitro. Treatment of fresh articular cartilage with a proteolytic enzyme rendered the tissue active as a platelet aggregant. In vivo experiments demonstrated that surgical scarification of rabbit articular cartilage does not result in the adhesion of autologous platelets.

The proteolytic enzyme may affect the core protein of the proteoglycan subunit which may interact with the collagen molecule, thereby making crucial sites accessible to platelets. Intact or lacerated cartilage does not support thrombus formation. On the other hand, after the complete extraction of the proteoglycan matrix, cartilage collagen induced platelet aggregation. This process appeared to involve the formation of periodic bridges between the platelet membranes and the dense bands of the collagen fibrills (Havdrup and Telhag 1977). The role of collagen in platelet adhesion and aggregation has been a subject of intensive research for more than a decade. Purified cartilage collagen proved itself to be fully active as a platelet aggregant. Addition of small amounts of the proteoglycan subunit bloked platelet aggregation, whereas chondroitin sulfate, a major glycosaminoglycan component of cartilage matrix, impaired platelet aggregation, only at concentrations which resulted in a marked increase in viscosity. The possibility that platelets may also serve a crucial function in connective tissue repair has sometimes been raised but has never been pursued in depth.

It is generally recognized that irreversibly aggregated platelets form a firm substrate for the deposition of fibrin as well as for the proliferation of fibroblasts. The nature of the intermembranous bond between individual platelets, or the mechanism whereby platelets adhere to collagen, is possible that a modification of the cartilage matrix would permit platelets to interact with cartilage collagen in vivo. Platelet-derived factors are released which set the stage for cartilage regeneration and enhance the proliferation of fibroblasts and other connective tissue cells.

If the hypothesis of chalones is applied to our result, the inhibition of mitotic activity of hyaline cartilage cells by chalones is not the decisive factor for the missing cartilage repair, as we found no signs of regneration in the group treated only with trypsin and not with blood. Houck and Hennings (1973) have found that extracts of either the medium from cultured human fibroblasts or of the cells themselves possess a trypsin-labile and thermolabile material with a molecular weight between 30,000 and 50,000 daltons. But we do not confirm the evidence for the existence of this chalone by the fraction and purification method. This observation suggests that proteoglycans may inhibit the wound healing process in cartilage. In considering the application of these facts to injuries of hyaline articular cartilage, it does not allow any clinical conclusion. Long-term resutls and further experiments are necessary.

ACKNOWLEDGEMENT

The advice of Dr. Jae-Young Hyun, ex-Lecturer, Orthopaedic Surgery, Yonsei University College of Medicine, was highly valued.

The authors wish to thank Mrs. Jeanie Choi for her excellent secretarial help.

REFERNECES

Bartholomew JS, Handley CJ, Lowther DA: The effects of trypsin treatment on proteoglycan biosynthesis by bovine articular cartilage. *Biochem J 227:429-437, 1985*

Boldingh WH, Laurence EB: Extraction, purification and preliminary characterization of the epidermal chalone;

A tissue specific inhibitor obtained from vertebrate skin. *Europ J Biochem 5:191-198, 1968*

Bullough WS, Laurence EB: The control of epidermal mitotic activity in the mouse. *Proc Roy Soc London Series B 151:517-536, 1960*

Farkas T, Bihari-Vanga M, Biro T: Thermoanalytical and histological study of intra-articular papain-induced degradation and reapir of rabbit cartilage. *Ann Rheum Dis 39:23-26, 1975*

Fuller JA, Ghadially FN: Ultrastructural observations on surgically produced partial-thickness defects in articular cartilage. *Clin Orthop 82:193-205, 1972*

Havdrup T: Trypsin-induced mitosis in the articular cartilage of adults rabbits. *Acta Orthop Scand 50:15-19, 1979*

Havdrup T, Henricson A, Telhag H: Papain-induced mitosis of chondrocytes in adult joint cartilage. *Acta Orthop Scand 53:119-124, 1982*

Havdrup T, Telhag H: Papain-induced changes in the knee joints of adult rabbits. *Acta Orthop Scand 48:143-149, 1973*

Houck JC, Hennings H: Chalones. *FEBS Lett 32:1-8, 1973*

Hunter W: On the structure and diseases of articular cartilages. *Philos Trans Roy Soc 42(B):512-521, 1743*

Landells JW: The reactions of injured human articular cartilage. *J Bone Joint Surg 398:548-562, 1957*

Mankin HJ: The response of articualr cartilage to mechanical injury. *J Bone Joint Surg 64-A:460-466, 1982*

Meachim G: Effect of scarification of articular cartilage in the rabbit. *J Bone Joint Surg 45-G:150-161, 1963*

Telhag H: Mitosis of chondrocytes in experimental osteoarthritis in rabbits. *Clin Orthop 86:224-229, 1972*

Thompson RC: An experimental study of surface injury to articular cartilage and enzyme responses within the joint. *Clin Orthop 107:239-266, 1975*

Weiss P, Kavanau JL: Chalones. *J Gem Physiol 41:1-47, 1957*

Wolf CR, Mankin H: The effect of experimental hemarthrosis on articular cartilage of rabbit knee joints. *J Bone Joint Surg 47-A:1203-1210, 1965*

Zudker-Franklin D, Rosenberg L: Platelet interaction with modified articualr cartilage. Its possible relevance to joint repair. *J Clin Invest 59:641-651, 1977*