

Changes in the Hyaline Articular Cartilage after Air Exposure

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The changes of hyaline articular cartilage from rabbits after air exposure were evaluated. The knee joints were exposed to air for periods of thirty minutes to two hours. The animals were killed periodically, at three days, one week and three weeks postoperatively. After sacrifice, the cartilage was removed and prepared for study by light microscopy and electron microscopy. Exposure to room air for thirty minutes produced chondrocyte necrosis in the upper third of the cartilage, and exposure for 60 minutes or longer produced chondrocyte necrosis of the entire thickness of articular cartilage at three days after arthrotomy. But, three weeks after arthrotomy, we could not find any chondrocyte necrosis in any rabbits at varying periods of air exposure. There was no significant change in proteoglycan content between the dried and control cartilage. Clinical Relevance: Exposing cartilage to air can cause transient and reversible cartilage damage. If these changes are not reversible, the orthopedic surgeon should consider avoiding the prolonged exposure of articular cartilage to air, since complete matrix disintegration is known to occur months after chondrocyte necrosis.

Key Words: Air exposure, articular cartilage

Articular cartilage is composed of relatively small numbers of chondrocytes, collagen and a hyaline matrix made up of proteoglycan molecules. The properties of articular cartilage depend on the structure and composition of the abundant extracellular matrix. Chondrocyte maintains the hyaline matrix by constantly degrading and synthesizing proteoglycan. The cells account for 5% or less of the tissue volume and do not have direct contact with each other. The interaction between the cells and the matrix is important.

Cartilage is a hyperhydrated tissue with values for water content ranging from 65% to almost 80% of the total wet weight. The remaining 20% to 30% of the wet weight of the tissue is principally accounted for by two macromolecular materials: collagen, which composes up to approximately 60% of the dry weight, and proteoglycan, which accounts for a large part of the remainder. Most of the water is freely exchangeable with both the synovial fluid and the blood stream. Only about 6% of the cartilage water is tight-

ly bound.

The preservation of cartilage and restoration of joint function are important problems in the management of joint disorders such as osteoarthritis, traumatic lesions and tumorous conditions. During the reconstructive surgery of knee joints, it is common for articular cartilage to be exposed to operating room air for a prolonged period. The tendency for the cartilage to dry out under these circumstances is increased. When articular cartilage is exposed to air for prolonged periods during arthrotomies, the cartilage becomes yellow, rough and loses its glossy appearance, resembling early degenerative joint disease. It is uncertain whether these gross changes precede the development of histologic changes associated with degenerative joint disease.

The purpose of the present study was to define the gross and microscopic changes after cartilage exposure to ambient air for a varying period of time, and to determine whether or not they are persistent and initiate degenerative joint disease.

MATERIALS AND METHODS

Mature New Zealand white rabbits, weighing three kilograms or more, were anesthetized with Nembutal (pentobarbital). The right knee of each animal was opened through a medial parapatella incision and the

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patella was dislocated. The skin and fascia were sutured back to create an opening that would not close spontaneously. The articular surface of the distal femur, including the articular cartilage of the trochlea, was exposed to room air for thirty minutes (12 animals), sixty minutes (12 animals) and two hours (12 animals). The left knee was opened and closed immediately, and was reserved as a control. All animals received an intramuscular injection of Kanamycin (0.16 gm/kg) for three days as prophylaxis against infection. Twelve animals were killed by intravenous injection of sodium pentothal at each of the following intervals: three days, one week and three weeks.

Immediately after death, a full thickness sample of cartilage, measuring approximately five by five millimeters, was removed from the femoral articular surfaces. It was necessary to remove the cartilage together with some fragments of subchondral bone. Each sample was divided into two blocks, and the blocks were immediately fixed and embedded for light and electron microscopy, as will be described. At least 5 slices were obtained from each block. They were fixed overnight in formalin, embedded in paraffin after decalcification and sectioned with a microtome. Some of these sections were stained with hematoxylin and eosin, and alcian blue (pH 2.5). The remainder were used to prepared for electron microscopy. They were fixed in 2 percent glutaraldehyde in paraformaldehyde and 0.1M cacodylate, pH 7.4, for two hours; washed in 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 (Silberbery *et al.* 1964; Warshawsky and Moore 1967; Weiss and Rosenberg 1968; Fuller and Ghadially 1972; Shepard and Mitchell 1976). The sections were stained with uranyl acetate and lead citrate and were examined with a Hitachi H-500 electron microscope.

RESULTS

The articular cartilage of the trochlea was evaluated in 36 femurs. In each evaluation, the right trochlea was compared with the left which served as the control.

Gross Evaluation

A distinct color change was seen in the exposed cartilage at 3 days after open arthrotomy. The cartilage was yellow-brown, and in some instances, gentle undulation could be seen. The duration of exposure to

ambient air did not affect the gross appearance of articular cartilage. The colour change was less apparent in the exposed cartilage at 7 days and 3 weeks after open arthrotomy. The control side had no colour changes. The mechanism responsible for these changes is unknown. A water content may be involved, although that possibility was not determined. Seemingly, the gross changes seen in articular cartilage exposed to air are transient.

Histologic Evaluation

According to the surface integrity, necrosis, distribution pattern of chondrocytes, cellularity and neovascularization of uncalcified cartilage, a histologic evaluation was done. Three days after thirty minutes of drying, the cartilage cell had lost its normal contour in the upper third of the entire thickness.

But the signs of necrosis of the chondrocytes did not extend beyond the deep radial zone, and often affected only about one-half of the cartilage in two specimens. A statistical difference did not exist in the mean total cell count, distribution pattern of chondrocytes and neovascularization. At one week after drying for thirty minutes, there was loss of columnization of cellular distribution, cloning of chondrocytes and hypercellularity. The previous necrotic changes of the chondrocytes were still noted, but they were patchy, and usually extended to the deep radial zone (Fig. 1). At three weeks after drying for thirty minutes, chondrocyte necrosis was not seen.

Three days after sixty minutes of drying, the chondrocyte necrosis was extended to the entire layer. At one week after open arthrotomy for sixty minutes, there was loss of columnization of cellular distribution and cloning of chondrocytes. The previous necrotic changes of chondrocytes were still noted, but there were regional reparative areas in the articular cartilage (Fig. 2). Chondrocyte necrosis was not seen at three weeks after open arthrotomy for sixty minutes. Three days after two hours of open arthrotomy, necrotic change of chondrocytes was observed in the entire articular layer. After three weeks after open arthrotomy for two hours, there was no chondrocyte necrosis in the entire layer.

The proteoglycan content was evaluated subjectively based on the staining intensity of alcian blue, because proteoglycan molecules have an affinity for such stains. A decrease in proteoglycan content is reflected by a decrease in staining intensity. In this study, differences in staining intensity with alcian blue could not be seen between the left and right trochlea in any group.

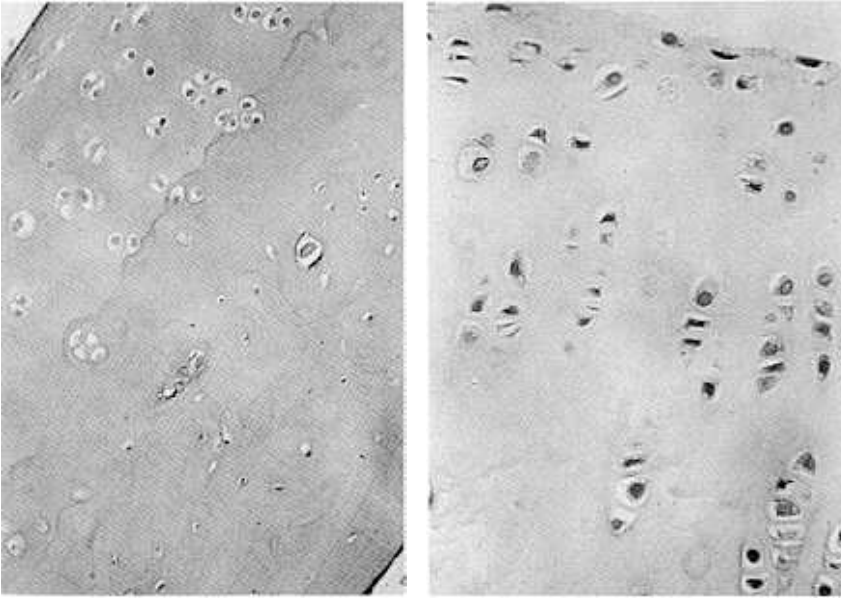


Fig. 1. Light microscopic finding of the rabbit cartilage, after air exposure for 30 minutes.

- a. Three days after exposure, pyknosis of the chondrocytes is noted in the upper third of the cartilage (H & E, $\times 200$).
- b. Seven days after exposure, a patchy-shaped necrotic area in the superficial layer of the articular cartilage is still noted (H & E, $\times 400$).

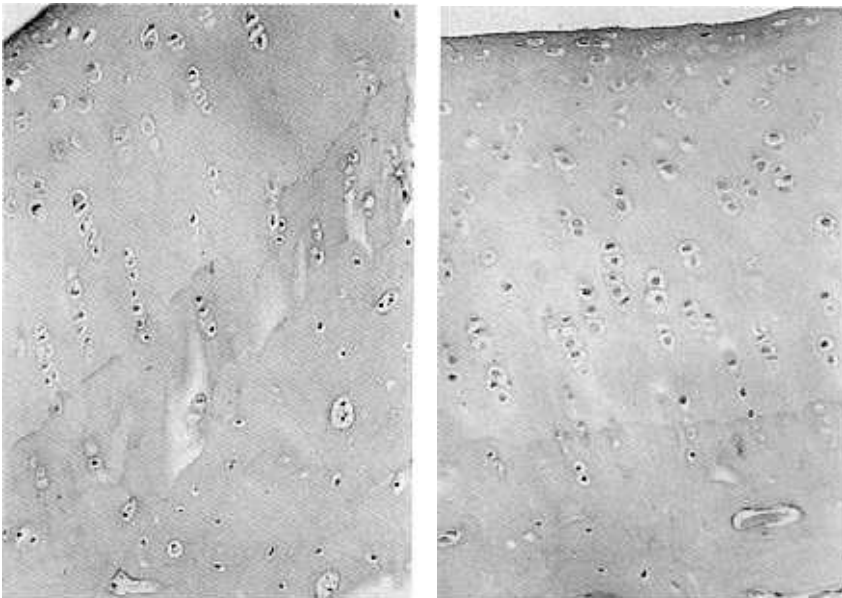


Fig. 2. Light microscopic finding of the cartilage, after air exposure for sixty minutes.

- a. Three days after exposure, the entire thickness of articular cartilage shows pyknosis and cellular disruption (H & E, $\times 200$).
- b. Seven days after exposure, the necrosis of the chondrocytes is patchy and extends to the deep radial zone. Hypercellularity is noted (H & E, $\times 200$).

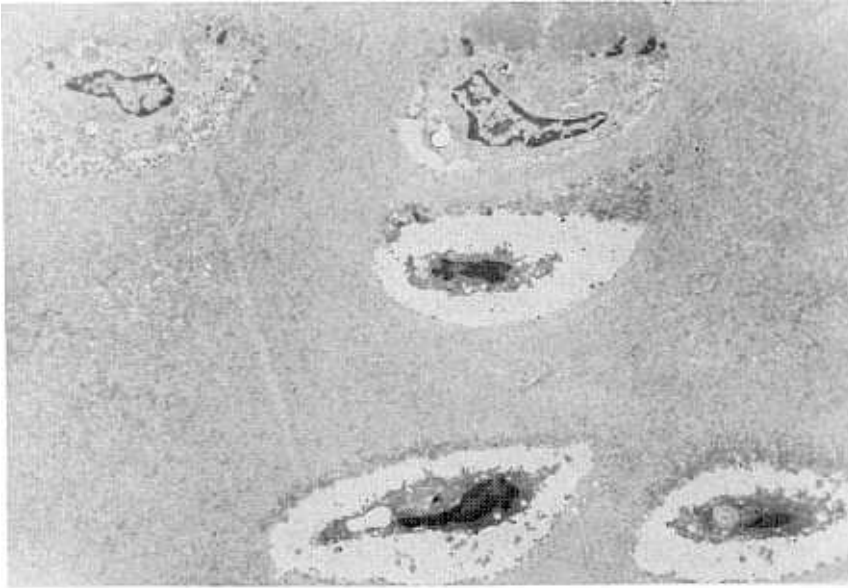


Fig. 3. Three days after thirty minutes of drying, an electron photomicrograph of the upper third of the cartilage shows disruption of cellular contents, and cellular retraction from the matrix ($\times 5,000$).



Fig. 4. Three days after sixty minutes of drying, the necrotic change of the chondrocyte was extended to the deep layer of cartilage ($\times 5,000$).

Electron Microscopic Evaluation

Electron microscopic findings confirmed the results

of the histologic evaluation. In electron microscopy of cartilage that had dried for thirty minutes, characteristic changes in the necrotic cells could be seen in the upper third of the cartilage. All of the

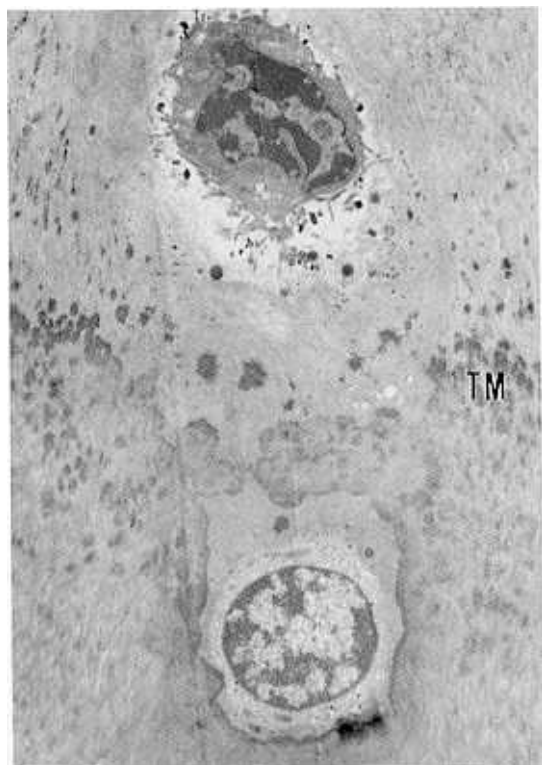


Fig. 5. Seven days after sixty minutes drying, necrosis of the chondrocyte above the tide-mark of the rabbit cartilage was still noted ($\times 7,500$).

TM: Tide mark

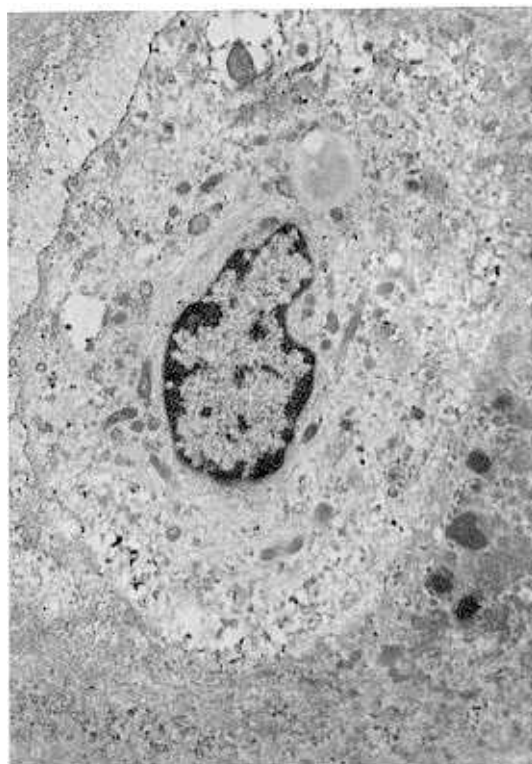


Fig. 6. Three weeks after one hundred and twenty minutes of drying, the normal chondrocyte of the intermediate layer was seen ($\times 12,000$).

necrotic chondrocytes had retracted from the matrix, the cell membranes had ruptured, and the nuclei and cytoplasmic organelles had condensed into an electron-dense, featureless mass. Electron-dense bodies, acceptable as lysosomes on a morphological basis, were more frequently encountered than normal. It showed early degenerative changes such as an increase in the number of intracellular filaments and a decrease in the amount of rough endoplasmic reticulum and Golgi apparatus (Fig. 3).

In the entire thickness of articular cartilage that had been exposed to room air for sixty minutes or longer, the nucleus of the chondrocyte had lost its chromatin network detail and had retracted from the cytoplasm. This change was noted one week after open arthrotomy in all groups (Fig. 4, 5). At three weeks after drying of the surface of the joint, the signs of necrosis were absent in the groups (Fig. 6). The chondrocytes showed a relatively large cytoplasmic volume, an elaborately developed granular endoplasmic

reticulum, and an extensive Golgi complex, which is characteristic of cells actively engaged in protein synthesis.

DISCUSSION

The articular cartilage lacks blood vessels, lymphatic vessels and nerves; and once formed, it appears to remain unchanged unless it deteriorates. It distributes the load to reduce the maximum amount of stress applied to the subchondral bone, and it can be deformed and rapidly regain its original shape. The articular cartilage participates in joint lubrication, and provides an unequaled low friction bearing surface, although only a few millimeters thick, with a limited capacity for repair.

The content of articular cartilage is extraordinarily important in maintaining the resiliency of tissue, as well as contributing to the almost frictionless movement associated with a boundary lubrication system. The

water content is highest at birth, with only a modest decrease in adult tissue, and a further slight diminution with advanced age. An increase in total water content has been noted with immobilization and with joint motion without weight bearing. Any interference with articular cartilage may lead to regressive changes in the cartilage (Meachim 1963; Mankin *et al.* 1971; Telhag and Lindberg 1972; Vignon *et al.* 1974).

Orthopedic procedures expose articular cartilage to operating room air for varying periods of time. During some ligamentous procedures, this may be as long as two hours. There are two harmful effects to articular cartilage after air exposure (Mankin 1974; Bauer *et al.* 1986; Mitchill and Shepard 1989). The first is dryness of cartilage. And the second is nutritional deficiency of cartilage through the synovial fluid during the air exposure.

Articular cartilage is considered to be a hyperhydrated tissue. Its water content is variously estimated as 60 to 80 percent of the total weight. The mechanism of water binding within the cartilage is not entirely understood.

Because most of the extracellular matrix consists of collagen and proteoglycan, and gel formation occurs, then water is in contact with either of these macromolecules. The water of the gel, although unable to flow, is believed to be freely exchangeable with that of fluids on the other side of the gel membrane, and is subject to all the physicochemical laws that govern osmotic solutions or membrane theory. However, little is known about the gross exchange of water between the articular cartilage and the synovial fluid. Only a small portion of the water (5%) remains tightly bound, and it cannot be removed by heating or dessication.

The articular cartilage obtains its nutrition from the diffusion of joint fluid and transudation from the subchondral vessels in the deep area (Honner and Thompson 1971). Synovial fluid, the joint lubricant, is composed mainly of hyaluronate, an extremely large polymerized sugar molecule, and a mixture of proteins and electrolytes which differ slightly from those of serum. It also has been variously proposed that insufficient mechanical circulation of the fluid plays a role in the pathogenesis of osteoarthritis. The controversy is the extent to which this source is supplemented by the subchondral route. The clinical observation, that loose cartilaginous bodies within the joint grow in size, has emphasized the importance of synovial fluid as the principal source of nutrition for articular cartilage. The prevailing view is that the synovial fluid is by far the most important source of nutrients for joint cartilage. There is minimal nutritional exchange

between the epiphyseal bone and articular cartilage at any age. Therefore, air exposure of hyaline cartilage may induce the development of histologic changes associated with degenerative joint disease.

This study revealed that the articular cartilage exposed to room air for as little as thirty minutes showed chondrocyte necrosis in the upper third of the rabbit's articular cartilage. This change was even more advanced to total destruction of cartilage cells throughout all layers of the cartilage after sixty minutes of exposure.

At one week after exposure for sixty minutes or longer, previous chondrocyte necrosis was still noted. But the necrosis was not seen in all rabbits for varying periods of air exposure.

It may be argued that the articular cartilage of the knee of a rabbit is considerably less thick than that of the knee of a human. Application of information from the studies of animal articular cartilages to the treatment of human joint injuries depends on the understanding of the similarities and differences between human and animal articular cartilage (Arnoczky and Marshall 1981; Mankin 1981). Differences in cartilage thickness existed in all regions of the joints, and presumably reflected differences in the loading due to the species' differences in posture, gait and weight. Rabbits habitually rest with their knees flexed 140° and even when hopping, they rarely extend their knees to less than 100° of flexion. The overall species' differences in cartilage thickness and composition may be responsible for differences in the results of cartilage injuries and diseases. All rabbits were clinically normal before surgery and were allowed unrestricted joint motion after surgery.

In clinical situations, the necessity of arthrotomy often indicates preexisting joint diseases. Postoperative joint immobility may also be required. The effects of exposure to ambient air on articular cartilage in these situations may be more severe. It is unknown how much greater the depth of destruction might have been if the cartilage had been thicker.

The proteoglycan content was evaluated subjectively based on the staining intensity of alcian blue, because proteoglycan molecules have an affinity for alcian blue. A decrease in proteoglycan content is reflected by a decrease in staining intensity (Gauer *et al.* 1986). When comparing the right trochlea with the control, there was no change in staining intensity, indicating that there was no significant change in proteoglycan content between the dried and control cartilages.

Seemingly, exposing cartilage to ambient air, with the gross and histologic changes being transient, caus-

ed reversible cartilage damage, although the results of an experimental study may differ from clinical situations. This study clearly indicated that drying is harmful to the chondrocytes of articular cartilage and should be studiously avoided in the operating room.

If these changes are not reversible, the orthopedic surgeon should consider avoiding the prolonged exposure of articular cartilage to the open air, since complete matrix disintegration is known to occur months after chondrocyte necrosis.

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