Healing of Articular Cartilage in Intra-Articular Fractures in Rabbits*

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ABSTRACT: **Intra-articular fractures were created in the distal end of the femur in adult rabbits. Fractures were then reduced in three different fashions: incomplete reduction, adequate reduction without compression of the fragments, and ideal reduction, which consisted of reduction with strong compression of the fracture fragments. Animals were killed at intervals ranging from seven weeks to one year, and the cartilage fractures were studied by light, transmission, and scanning electron microscopy, using proteoglycan stains.**

Cartilage fractures that were inadequately reduced or were adequately reduced without compression healed by fibrocartilage only. Fractures that were reduced with compression across the fragments healed with a tissue which, by light and electron microscopy, appeared to be hyaline cartilage, which showed the collagen and proteoglycan orientation and relationships of normal hyaline cartilage. The healing of these defects appears to be the result of a proliferation of cells emanating from the region of the tidemark, and perhaps of migration of chondrocytes from the surface into the lesion. We postulate that compression of the cartilage surfaces either creates a physical environment that allows certain chondrocytes to heal the defect with hyaline cartilage, or, by coapting the surfaces, prevents ingrowth of granulation tissue from the subchondral bone that might interfere with repair by hyaline cartilage.

This study suggests a hitherto unappreciated capacity for hyaline cartilage to repair structural defects.

CLINICAL RELEVANCE: **Accurate reduction of intra-articular fractures with interfragmentary compression may create the environment whereby cartilage cells may repair the cartilage fracture — a capacity not previously thought possible in adult cartilage. The repair of cartilage defects provides obvious benefits to the patient.**

The repair of defects in the structure of articular cartilage depends on the maturity of the animal and the type of defect. Several authors have shown that the healing of incomplete defects does not occur in mature animals and occurs only rarely in immature animals[1-4]. We have recently shown that repair of full-thickness defects — those created by drill holes through denuded femoral condyles into the subchondral bone — occurs by ingrowth of granulation tissue from the marrow. Initially this tissue undergoes metaplasia to form hyaline cartilage, but later it becomes fibrocartilage and one year later it appears somewhat degenerated[5]. What, then, is the potential for cartilage repair in the full-thickness defects created by intra-articular fractures, when the fracture has been ideally reduced and immobilized? We created intra-articular fractures in mature rabbits and contrasted the results of cartilage repair in those that were ideally reduced and immobilized with those that had less than ideal reductions. The results of this study constitute the substance of this report.

Materials and Methods

Intra-articular fractures were created in the distal medial femoral condyle in twenty-eight mature rabbits (Fig. 1). Following the creation of this consistently reproducible lesion, each fracture was reduced and immobilized with an AO navicular lag screw. In some of the fractures the screw was tightened only sufficiently to approximate the fracture fragments (Fig. 2). In others strong compression was applied to the fragments, as the dense cortical nature of rabbit femoral bone makes it possible to achieve rather large compression forces in these fractures. Postoperatively, the rabbits were returned to normal cage activity and quickly regained full use of their limbs.

Groups of four to six animals were killed at intervals from seven weeks to twelve months following fracture. When they were killed, the joints were photographed and specimens were prepared as follows. The fractured distal end of the femur was removed and fixed in 2 per cent buffered glutaraldehyde containing either 0.2 per cent ruthenium red or 0.1 per cent toluidine blue 0, as described previously[6,7]. The fractured femur was then decalcified in 4.13 per cent EDTA, pH 7.4[8]; trimmed into small blocks containing the fracture; rinsed in buffer; and fixed in 2 per cent osmium tetroxide containing either 0.05 per cent ruthenium red or 0.025 per cent toluidine blue 0. The ruthenium red-fixed cartilage was additionally stained with p-phenylenediamine during the dehydration phase, as we have reported previously[7]. During embedding in Spurr resin, the blocks were oriented so that full-thickness sections of the fracture could be cut for visualization by either light or electron microscopy. The sections to be studied by transmission electron

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FIG. 1

FIG. 2

FIG. 3

Fig. 1: A complete fracture before reduction.
Fig. 2: Only a slight separation remains in the cartilage after reduction of the fracture with an AO navicular screw.
Fig. 3: When reduced with minimum pressure, fracture sites united with an ingrowth of new tissue (arrow).

microscopy were also stained with uranyl acetate and lead citrate and were examined with a Phillips 400 transmission electron microscope.

Several joints were removed at six and twelve months, fixed, critically point-dried, coated with gold-palladium, and studied with a Phillips scanning electron microscope.

Results

Seven weeks after reduction without compression, a broad area of whitish tissue could be seen filling the defect between the fracture fragments (Fig. 3). By light microscopy, it was noted that the subchondral bone had united but the defect between the cartilaginous surfaces was filled by highly cellular tissue (Fig. 4). Although many of these cells had the appearance of chondrocytes by light microscopy, their electron microscopic appearance was that of fibrocytes. The cells were stellate in appearance, the organization of collagen was irregular, proteoglycan was deficient about them, and there were no cytoplasmic footlets. Seven weeks after reduction of fractures with maximum compression, the cartilage fracture was barely visible on inspection of the joint (Fig. 5). Histological examination revealed that the hyaline cartilage defect had been united by a cluster of cells whose maximum activity arose from the tidemark and by migration of cells at the surface of the cartilage into the fracture (Fig. 6). The electron microscopic examination of the cells at this time, however, suggested that fibrochondrocytes rather than hyaline chondrocytes were filling the defect.

On inspection of the joint three months after reduction *without* compression, a similar whitish material could be seen occupying the site of the old cartilage fracture. By both light and electron microscopy, this material had the appearance of fibrocartilage. However, three months after reduction and strong compression, no sign of the old frac-

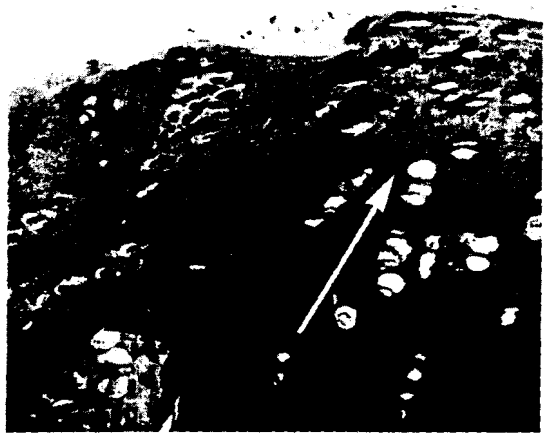

FIG. 4

FIG. 5

Fig. 4: The new tissue in a fracture reduced with minimum pressure bears no resemblance to the original adjacent joint cartilage (arrow) (× 234).
Fig. 5: Following maximum compression, the fracture is barely evident (arrow).

FIG. 6

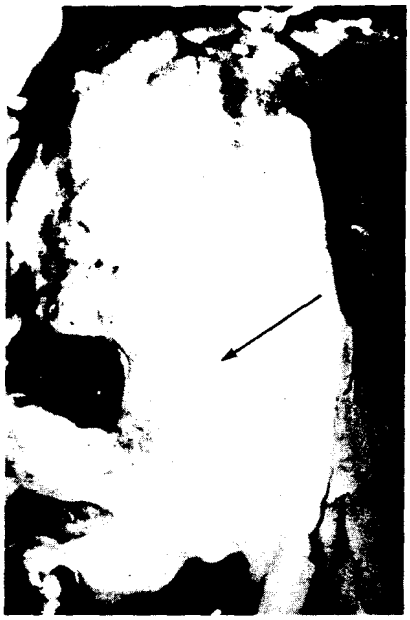

FIG. 7

FIG. 8

Fig. 6: Fewer cells filled this fracture, which was more ideally reduced. The cells appeared to emanate (arrow) from the area of the tidemark (tm) (× 650).

Fig. 7: Three months after reduction and maximum compression, the fracture site (arrow) cannot be detected.

Fig. 8: In the fractures that were ideally reduced by maximum compression, by light microscopy only a slight seam could be seen at the site of the fracture (arrow). Within the zone of calcified cartilage below the tidemark (tm) there are numerous cells (asterisks) characteristic of this region (× 635).

ture line could be seen on the cartilaginous surface (Fig. 7). Histologically, the subchondral bone was healed and the cartilage matrix defect appeared to have repaired, with some residual cellular activity noted just above the tidemark (Fig. 8). At the surface of the defect, elongated cells similar to those of normal hyaline cartilage were found. Deeper in the matrix, at the junction of the old cartilage matrix with the new reparative tissue, only a thin seam could be seen separating the new and the old material. Higher magnification of the area showed that the col-

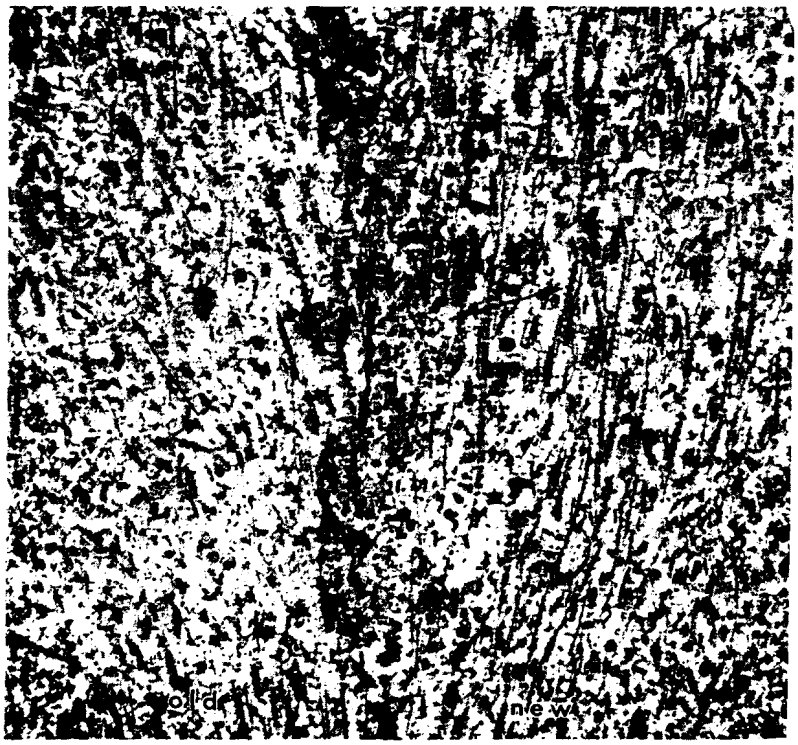

FIG. 9

Ruthenium-red-positive proteoglycan (arrows) was present between old and new collagen (× 18,535).

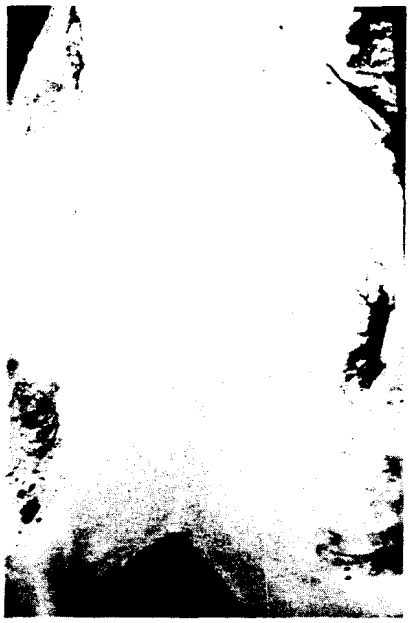

FIG. 10

In fractures that were ideally reduced by maximum compression, no sign of the fracture could be detected at six months.

lagen pattern on both sides of this seam appeared similar. The ruthenium-red staining of the proteoglycan showed similar proteoglycan and collagen relationships in both new and old matrix (Fig. 9).

small depression, the floor of which was filled with collagenous bundles running tangentially in the same direction as the normal cartilage surface (Fig. 11). Transmission electron microscopy revealed the surface to be intact, and confirmed that the orientation of the collagen was the same as that of normal cartilage. An examination of the deeper zones of the defect revealed that the collagen bundles, although they were somewhat less compact in the repair cartilage, were oriented in a similar direction toward the surface. The ruthenium and proteoglycan stain demonstrated fine proteoglycan precipitates attached to the collagen fibers[6,7] in both the repair matrix and the normal cartilage (Fig. 12). Six-month specimens from fractures that had been reduced without compression were similar to the three-month specimens.

Of the four animals that were killed at twelve months, three showed an excellent repair of the cartilage. Scanning electron microscopy showed the defect as either a small heaped-up area or a small depressed area, with continuity between the new and the old matrix (Fig. 13). Transmission electron microscopy demonstrated a healed cartilage defect with a small seam of reparative tissue extending from the area of the tidemark to the surface (Fig. 14). Within this seam and adjacent to it were healthy-appearing chondrocytes (Fig. 15). In the region of calcified cartilage, below the tidemark, the impression was that cells had divided and migrated upward in the defect, carrying small

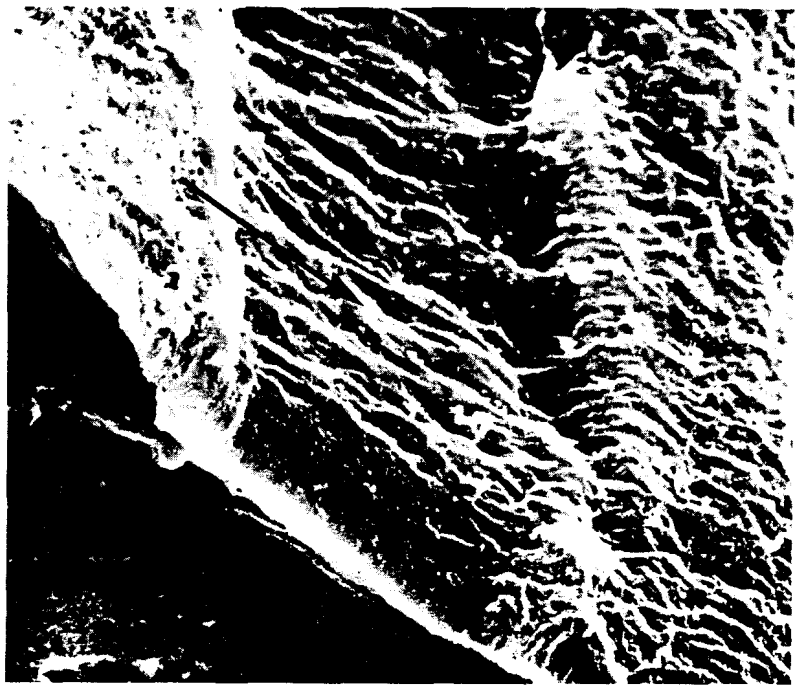

FIG. 11

Six months after injury, in fractures that were ideally reduced by maximum compression, as seen on scanning electron microscopy the orientation of collagen fibers at the surface of the reparative tissue (arrow) was similar to that of the adjacent, normal cartilage.

Six months after reduction with maximum compression, an examination of the joint surface again showed no evidence of the old fracture line (Fig. 10). By scanning electron microscopy, the surface of the defect showed a traces of the tidemark with them. Higher magnification of the area of cartilage repair demonstrated a normal-appearing relationship between collagen and proteoglycan (Fig. 16).

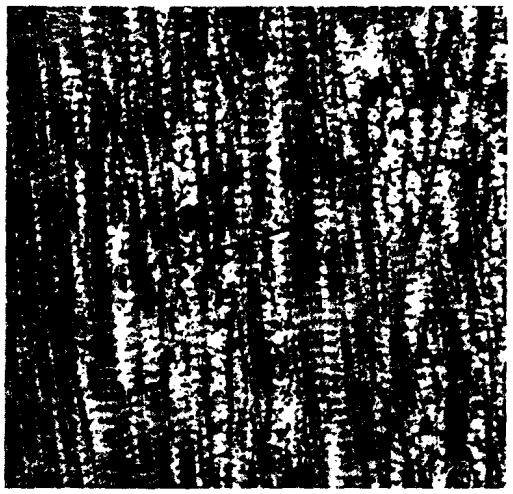

FIG. 12

FIG. 13

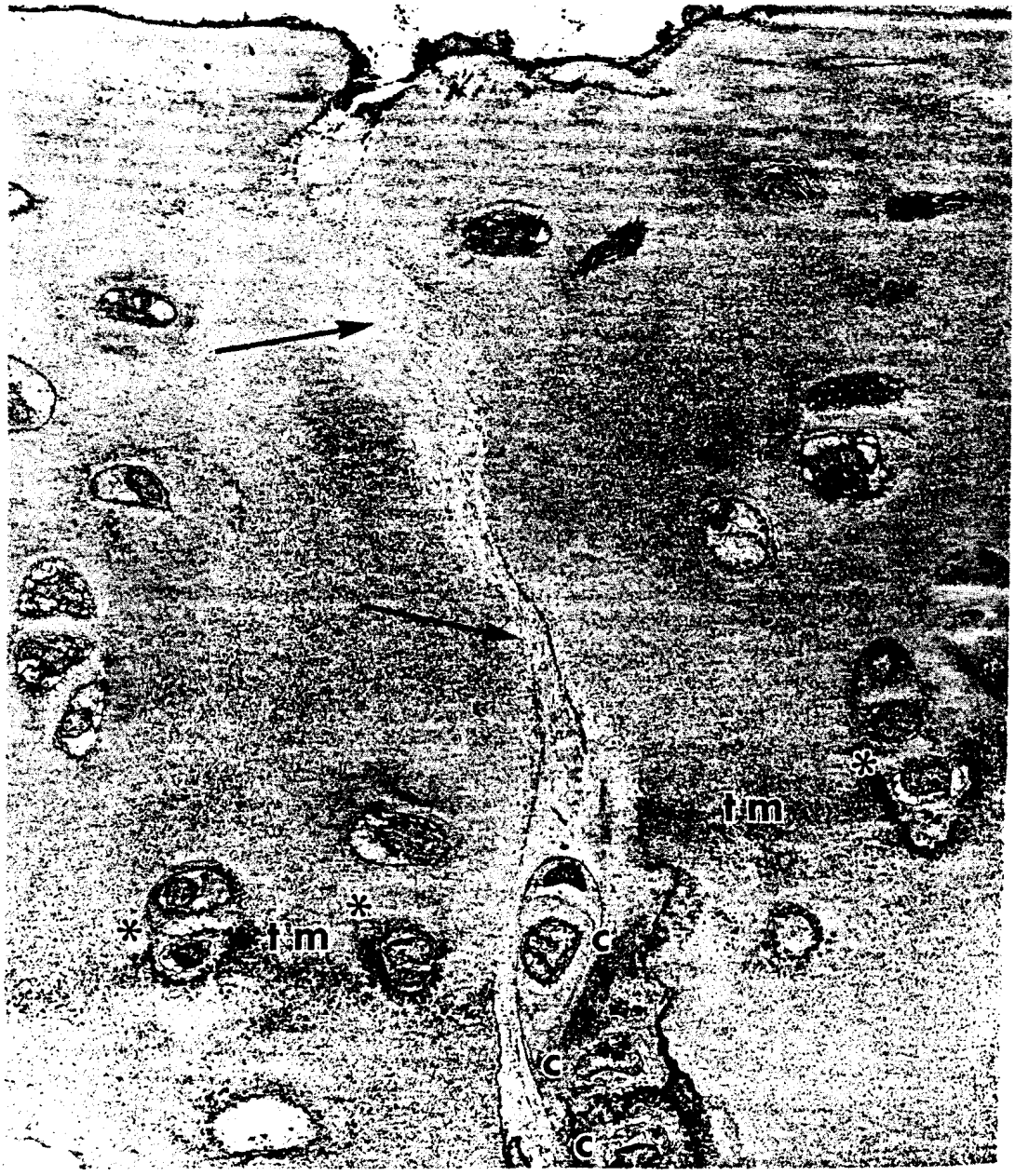

FIG. 14

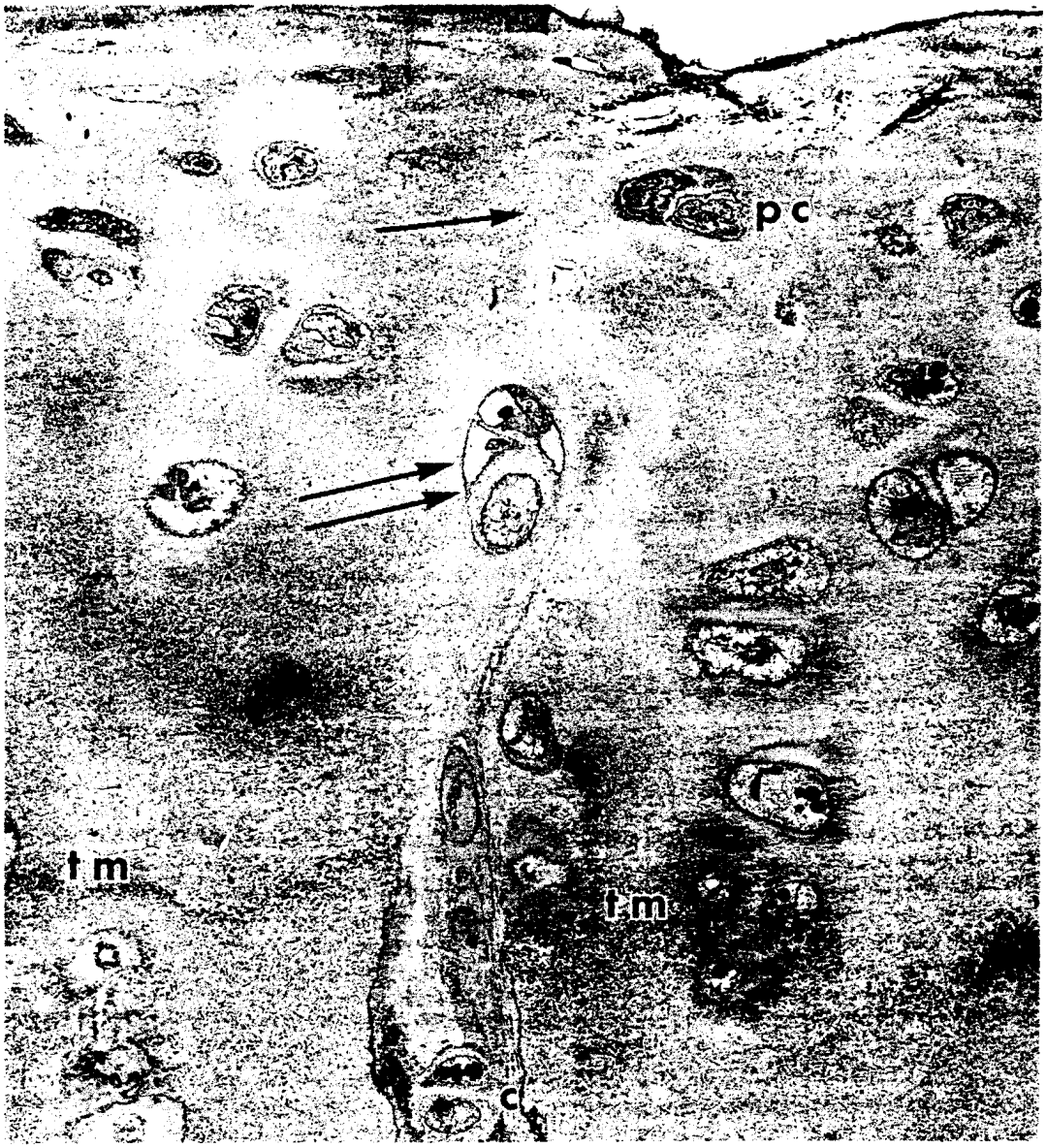

FIG. 15

Similarly healed fractures contained numerous chondrocytes (c) extending into the healed fracture at the tidemark (tm). A pair of chondrocytes (pc) can be seen just below the joint surface and adjacent to the new matrix (arrow), and also within it (double arrow) (× 1,320).

Discussion

At all stages of this study of mature animals, compression of the fracture fragments appeared to consistently produce a better repair of the cartilage articular surface than did reduction and immobilization without compression. In the latter fractures a fibrocartilaginous material appeared to fill the gap and there was little evidence that this had converted to hyaline cartilage. In the specimens that had had strong compression, a considerable amount of repair appeared to be taking place as early as seven weeks. The reparative tissue appeared to arise from activity in cells at the region of the tidemark. By three, six, and twelve months following compression of the fragments, both the matrix in the cartilage fracture and the old matrix outside it exhibited similar morphological patterns of collagen orientation and collagen-proteoglycan relationships. In addition, cells in the defect had the morphological appearance of hyaline chondrocytes. Although on occasion a small depression was seen in the surface by both light and scanning electron microscopy, in all successful instances

Fig. 12: The new matrix is slightly less compact than the original matrix (arrow) and proteoglycan is present between both collagens (× 39,965).
Fig. 13: By scanning electron microscopy, the edges of the defect (arrows) are seen to be in continuity with the fracture.
Fig. 14: A seam (arrows) of new cartilage matrix can be seen extending from the tidemark (tm) to the joint surface. Several chondrocytes (c) within the fracture appear to be emanating from the region of calcified cartilage. Chondrocytes in pairs (asterisks) extending into the cartilage are also visible bordering the tidemark (× 1,416).

FIG. 16

At twelve months abundant ruthenium-red-stained proteoglycan is seen between the collagen fibers of the fracture (arrows) (× 29,460).

of cartilage healing there was continuity of tangential orientation of matrix at the surface of the defect.

In our previous study, in which holes were drilled in the subchondral bone, a tissue grew into the drill-holes and initially repaired the defect in a fashion similar to that seen in the fractures in this study that were not rigidly compressed[5]. In that study, hyaline cartilage appeared initially, which then became fibrocartilage and eventually fibrillated.

It must be assumed that the compression of the fracture surfaces in this study is only temporary, and that these surfaces are not under continuous load for any significant period of time following the tightening of the screw. How, then, can compression mediate the changes described, and induce apparent hyaline cartilage repair? Two possibilities arise: first, forceful compression may so coapt the surfaces as to seal the subchondral bone and prevent the ingrowth of granulation tissue that was seen in the experiments in which drill-holes were created[5]. The absence of this granulation tissue and the initial force exerted by compression may produce a physical environment that induces chondrocytes at the tidemark to divide and migrate into the defect. Evidence for such migration perhaps is scanty, although there were suggestions that the cells as they migrated up into the defect were carrying along with them wisps of the electron-dense, amorphous material seen at the tidemark. The second possibility is that the physical environment created by compression may stimulate chondrocytes in the normal cartilage, close to the area of the fracture, to also participate in the repair. However, evidence for the migration of these cells into the defect was not seen.

It was previously thought that structural defects could not be repaired in mature cartilage. This report suggests that under certain conditions mature articular cartilage may undergo satisfactory repair. The only variable in this experiment was the degree of compression across the fracture surfaces. In what way this compression produces the proper biological requirements for repair is unknown. However, the possible relationship between the degree of compression of the surfaces of a cartilage defect and the quality of the repair of that defect may have important implications, not only for intra-articular fractures but also for other disease states involving articular cartilage.

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