A Study of Electrochemical Enhancement of Articular Cartilage Repair

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The question of the regenerative capability of mammalian articular cartilage has been under study for the past 200 years. From a clinical viewpoint, there is little doubt that the majority of major defects in articular cartilage do not heal satisfactorily. The defects heal with fibrous tissue which subsequently undergoes metaplasia to fibrocartilage.[11, 21, 26-28] Limited attempts at repair occur by proliferation of surviving hyaline cartilage cells [7, 8, 10, 12] or by metaplasia from bone marrow elements.[15, 29, 30] Mitosis and increased biochemical activity of hyaline cartilage cells subjected to osteoarthritic changes or chronic trauma[14, 16, 20, 22, 23, 32] and the capacity of hyaline cartilage to proliferate *in vitro*[13, 18, 19, 24] also constitute evidence of reparative power but whatever reparative or regenerative processes occur are usually overwhelmed by rapid proliferation of fibrous tissue. Thus, while hyaline cartilage is capable of regeneration, the process is so limited in extent that it is functionally unsatisfactory. Mankin and Lippiello[23] have suggested that changes could be effected in the micro-environment of the residual hyaline cartilage which might result in enhancement of its limited regenerative capability.

During the past decade this laboratory has been involved in a study of the electrical control systems regulating bone growth. It has been possible to describe in some detail the control systems involved in bone growth in response to mechanical stress[1, 4, 25] and in fracture healing.[5] The latter study led to a concept of the electrical control systems responsible for regenerative healing in general[2] and in a preliminary study, it was found possible to induce limited limb regeneration in mammals by simple electronic means[3, 6] While the regenerates were small in size, they were appropriately organized and multi-tissue in nature, including normal epiphyseal plates and articular cartilage. It seemed reason-

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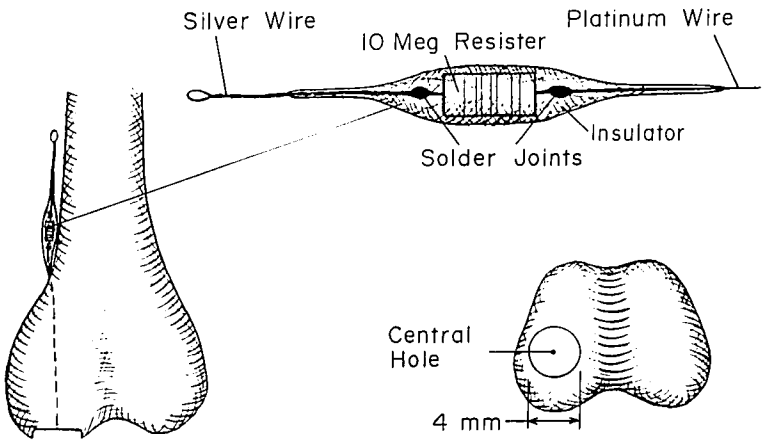


FIG. 1. A representation of the 10 megohm device (upper right), an anteroposterior view of the distal femur with the device in place (left) and a view of the articular surface of the condyles perpendicular to the longitudinal axis of the femur, depicting the 4 mm circular defect and the central drill hole (lower right).

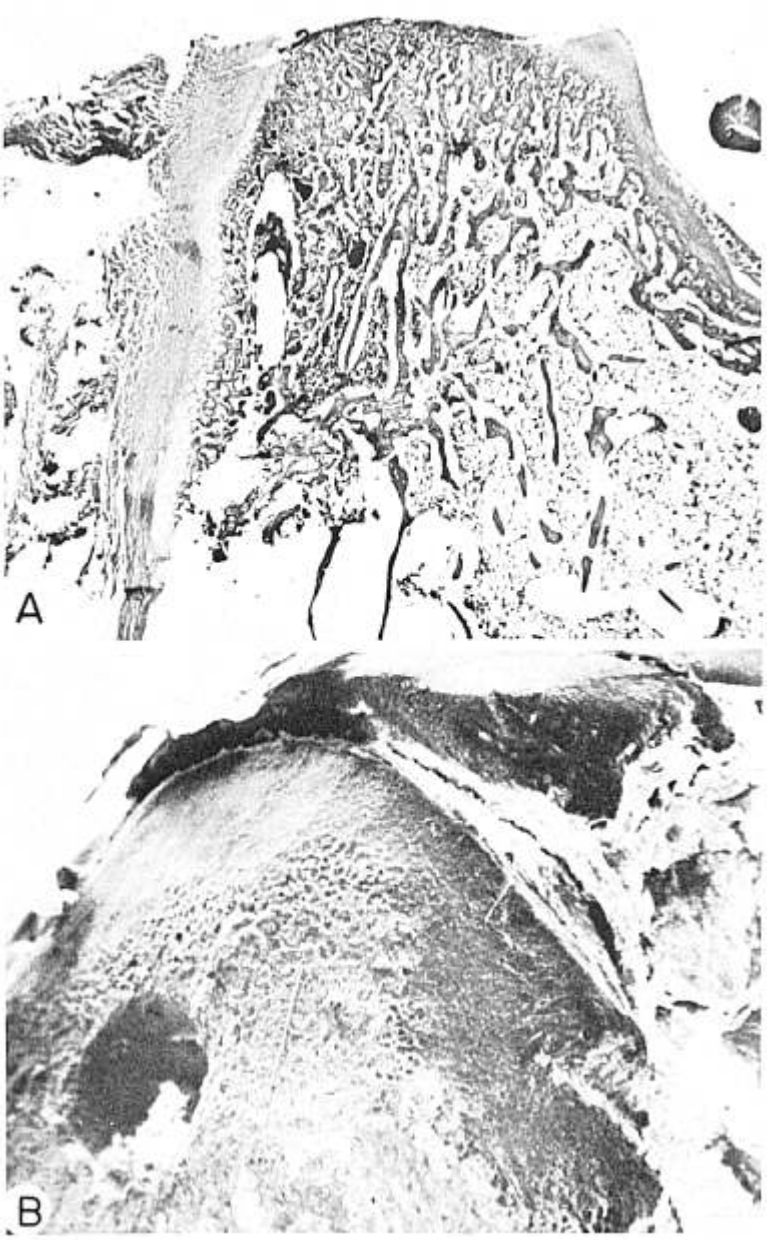
able, therefore, to conclude that the limited regenerative capacity of articular cartilage might be enhanced by restoring the appropriate electrical environment. This paper reports the results of a study based on this concept in which encouraging results were obtained.

MATERIALS AND METHODS

Six-week-old New Zealand white rabbits weighing 0.6 to 1.0 kg were shaved, sedated with Nembutal (30 mg per kg) intravenously and anesthetized using anesthetic grade ethyl ether by the open drop technique. The knee was prepped and draped. A lateral parapatellar incision was made extending proximally into the quadriceps mechanism. The patella was dislocated medially exposing the lateral femoral condyle. Two types of articular defects were created in the lateral condyle, both with cartilage removal extending down to subchondral bone. On 20 condyles, the area denuded extended from the lateral margin of the weight-bearing surface to the intercondylar notch and from the level of the patello-femoral groove to a point 3 to 4 mm from the posterior margin of the condyle. A standard 4 mm defect was created in the center of the weight-bearing portion of 26 additional condyles using a cork borer to outline the defect (Fig. 1). The cartilage was removed through subchondral bone to the margin of the defect. In both groups a retrograde 0.75 mm drill hole was made through the central portion of the defect exiting proximally over the flare of the lateral femoral condyle. A series of immediate postoperative specimens were evaluated by light microscopy for evidence of remnants of hyaline cartilage in the defect area and were found to be clean. One specimen was examined by scanning electron microscopy, and noted to have a clean, sharp margin with a defect surface composed of subchondral bone (Fig. 2).

The devices used as current sources in these experiments are similar to those that have been successful in stimulating partial regeneration in rat forelimbs[3,6] and in frog limbs.[31] Silver platinum galvanic couples were made with 10 megohm internal load resistors which served to limit current levels and reduce the rate of current decline with time (Fig. 1). *In vitro* and *in vivo* measurements of the electrical characteristics of such units indicated initial currents of about 100 na (nano-amperes), decreasing to about 5 na in 24 hours and reaching a steady state value of about 3 na by 48 hours after implantation. Interelectrode potentials at the same time equilibrated between 40 and 70 mv, the platinum electrode being positive. The 10 megohm load is close to optimum since higher values tend to lower equilibrium currents drastically as the platinum interfacial resistance (approximately 100 megohms) is reached and exceeded. It is also important to note that this system is driven by electrochemical reactions at the electrodes and that the platinum-solution interface is cathodic, emitting negative ions into the surrounding tissue.

In addition to the 10 megohm active couples, several silver platinum units were made with the electrodes insulated from each other by epoxy resin and silicone. These units have a very high internal resistance, hence, they pass currents that are several orders of magnitude



FIGS. 2A and B. (top) Immediate postoperative 4 mm specimen with hematoxylin and eosin staining showing evidence of removal of articular cartilage to subchondral bone and sharp margins of the remaining articular cartilage. (15×) (bottom) Scanning electron micrograph of an immediate postoperative 4 mm defect demonstrating sharp articular margins superiorly and cancellous bone at the base of the defect with a central drill hole. (27×)

lower than the 10 megohm type. They maintain an interelectrode potential of about 300 mv.

All devices were insulated with room cure epoxy and coated with medical grade silicone, leaving only the outermost 2 mm of platinum and 6 to 8 mm of silver free to contact body fluids. Wire of 99.99 per cent purity and 0.25 mm in diameter was used for both metals. Cleaning and gas sterilization preceded implantation.

The devices were inserted through the drill hole with 2 mm of uninsulated platinum wire flush with the joint surface (Fig. 1). The resistor remained flush with the flare of the condyle while the proximal looped end was free, deep to the quadriceps mechanism. The re-

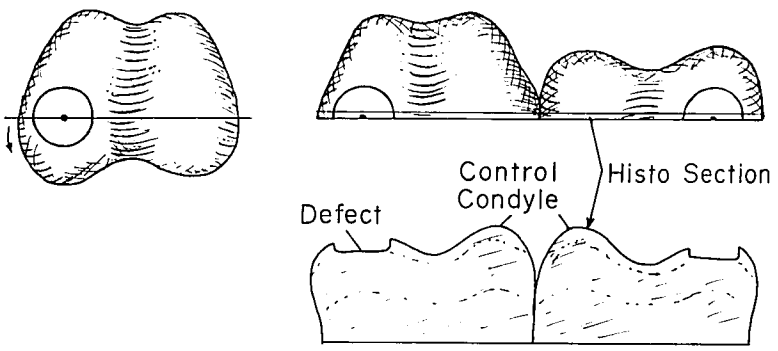


FIG. 3. A representation of the specimen showing the plane of sectioning after preparation (left). A hinge of tissue at the margin of the medial condyle remains to allow opening of the specimen and subsequent blocking in paraffin prior to sectioning with a microtome. The defect and the unoperated medial femoral condyle can be seen on each slide.

sistor was sutured to the periosteum with a single silk suture. The wounds were then closed with interrupted three-0 silk sutures after reduction of the patella. The control defects were produced similarly with no device inserted into the drill hole. Single metal wires were not used as control devices because of the possible electrochemical activity.[9] The animals received 100,000 units of procaine penicillin postoperatively and were fed rabbit chow and water *ad libitum*.

The femoral condyles were recovered at intervals from one to 9 weeks. The specimens for light microscopy were placed in unbuffered formalin and then decalcified with formic acid and sodium citrate. They were then dehydrated, imbedded in paraffin, and sectioned at 7μ, perpendicular to the weight-bearing surface of the condyles, in the frontal plane, with both condyles appearing in each section (Fig. 3). Slides were made of every tenth section and stained with either hematoxylin and eosin or toluidine blue.

Specimens for the scanning electron microscope were prepared by fixation with cacodylate buffered 5 per cent glutaraldehyde and dehydration with serial graded solutions of ethanol to 100 per cent. Processing continued with 50 per cent ethanol, 50 per cent amyl acetate, then 100 per cent amyl acetate with further drying in a Denton DCP-1 critical point dryer using liquid carbon dioxide. The specimens were glued to SEM stubs with conductive silver paint and coated wth palladium gold in a Denton vacuum evaporator. Viewing was done in a Cambridge Stereoscan scanning electron microscope (SEM).

Transmission electron microscope preparation was carried out with buffered 5 per cent glutaraldehyde and osmium tetroxide fixation and embedding in epon 8-12. Sectioning at 500 to 800 A was performed with a Porter-Blum MT-2 ultramicrotome. Staining with lead citrate and uranyl acetate was done prior to viewing with a RCA EMU 3D transmission electron microscope.

The reparative processes were evaluated and graded using the following categories.

A. *Marginal encroachment of hyaline cartilage over the defect site.* With 4 mm defects it was possible to quantitate the advancement of proliferating cartilage from the margin of the defect by gross inspection and microscopically by knowing the section thickness (7μ) and numerical frequency of sections stained (every tenth) giving 14 slides per mm of encroachment (Fig. 4), *i.e.*:

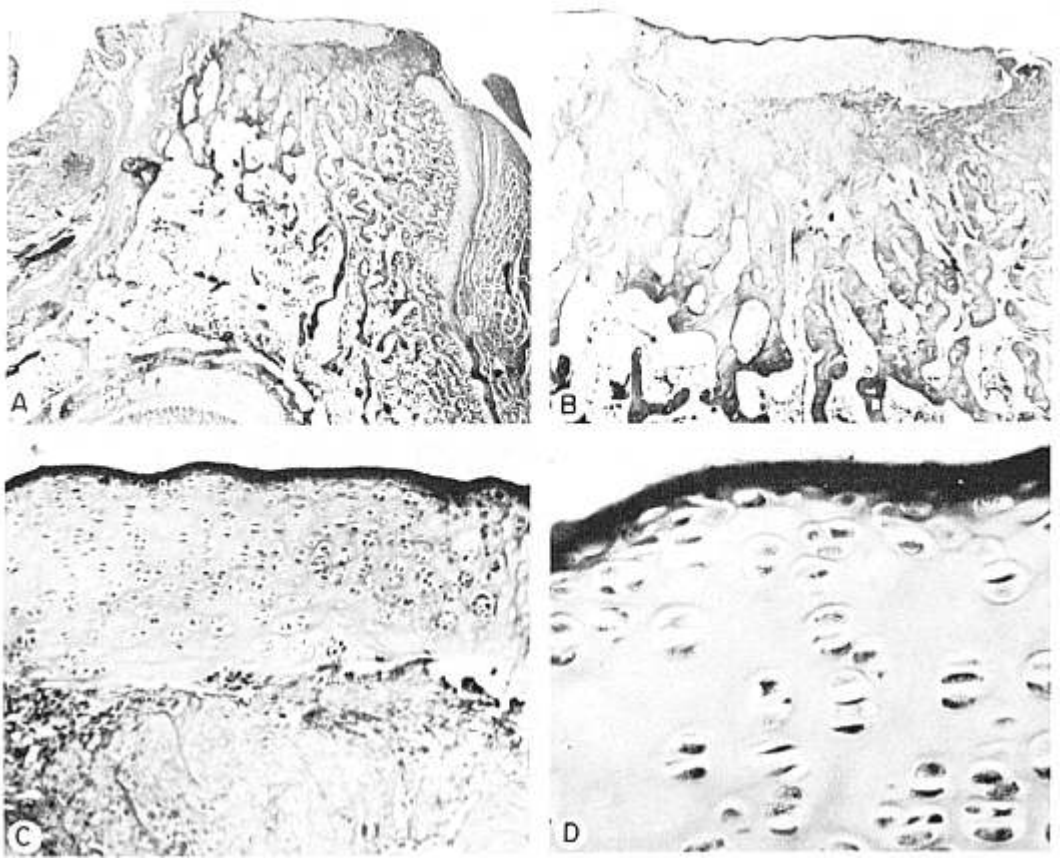
$$\frac{1\times10^{-3}}{(7\times10^{-6})}\times10=14 \text{ slides per mm encroachment.}$$

Grade 0 = no encroachment
Grade 1 = ½ mm encroachment
Grade 2 = 1 mm encroachment = 14 slides

B. *Marginal proliferative activity* — Hypercellularity, chondron formation, and the appearance of "flowing over" at the edge of the defect (Fig 5).

C. *Islands of cartilage* — Cartilage appearing unconnected to the margins graded on thickness and cellularity (Fig. 6).

D. *Central proliferation* — Proferation of subchondral elements in the vicinity of the central drill hole (Fig. 7).



FIGS. 4A to D. (A). 4 mm defect, with a 10 megohm device inserted for two weeks, revealing a cap of encroaching hyaline cartilage from the margin over a fibrous response from the subchondral bone. (×10) (B) The same specimen, ×25. (C) The same specimen, ×75, demonstrating proliferation with columnation of the cells. (D) The same specimen at ×150 showing homogeneous matrix and columnation of the cells, findings compatible with normal articular cartilage.

E. *Increased thickness of cartilage* — Cartilage thickness at the edge of the defect compared to normal thickness on the unoperated condyle (Fig. 8).

Each category was graded zero, 1+ or 2+ with zero representing no favorable response and 1+ and 2+ indicating increasing amounts of such activity.

RESULTS

LARGE DEFECTS

Controls. Gross inspection of the joints at 2 to 3 weeks revealed no evidence of wound infection with only occasional local mild tissue reaction around the sutures. The lateral margin of the lateral condyle appeared thick, and a yellow-white soft tissue response appeared in the defect. The drill hole was obliterated with the healing process. Light microscopy revealed central reactivity progressing from fibrous tissue to fibrocartilage as healing time progressed (Fig. 7). The drill hole was not seen, apparently obliterated with healing bone. The lateral margin revealed some cartilaginous proliferation of the thin, non-weight bearing rim with subsequent

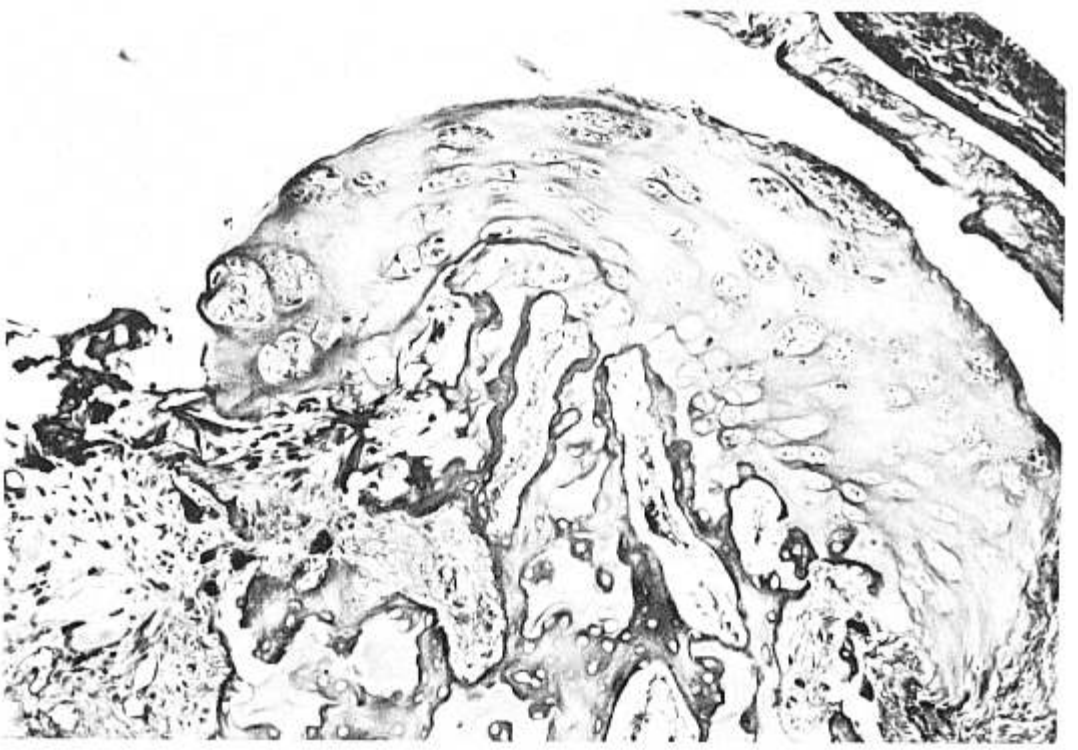


FIG. 5. A 4 mm defect 21 days after insertion of the 10 megohm device demonstrating marginal cellular proliferation and a flowing over of cells and matrix at the margin of the defect. (×125) 87%

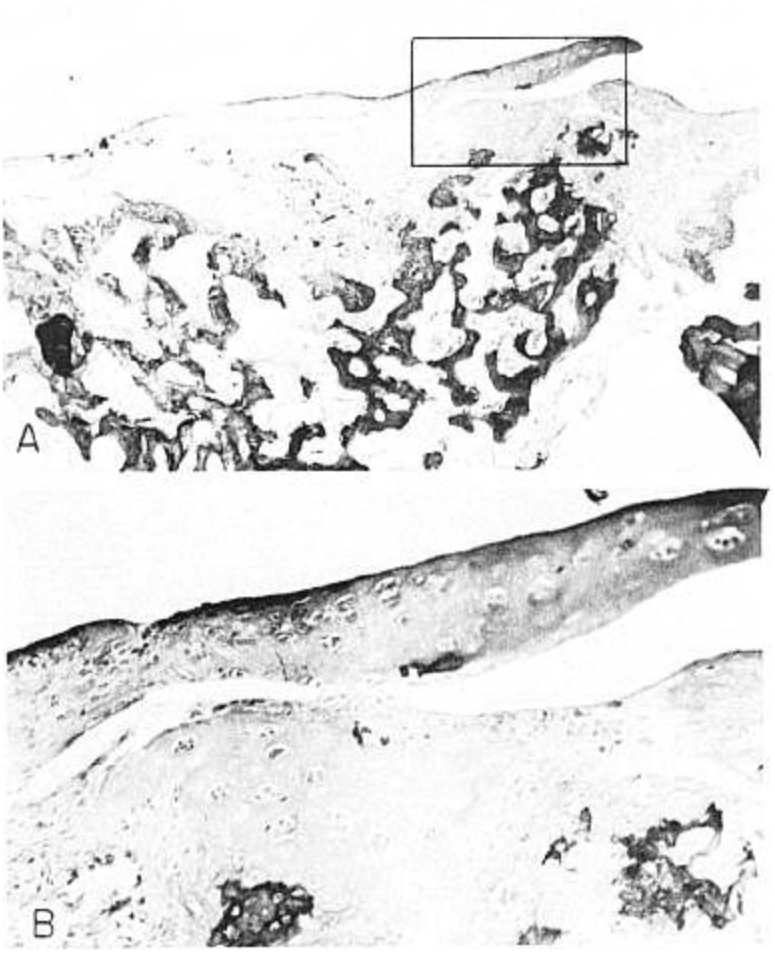
blending into fibrocartilaginous tissue (Fig. 9). There was some evidence of hypercellularity at the rim with little or no chondron formation.

Epoxy. There was no evidence of infection. However, 60 per cent of the devices were fractured at the epoxy-platinum wire junction. The gross and microscopic evaluation revealed changes similar in character and degree to the controls.

Ten Megohm. Gross examination revealed that the devices were undisplaced and intact, with no evidence of infection. The central region of the defect was filled with soft tissue similar to that seen in the controls. The lateral margin was frequently noted to have a thicker marginal lump than that noted in the controls (Fig. 9). Light microscopy revealed more frequent evidence of islands of fibrocartilage within the defect area than the controls and a greater degree of proliferation of the thin hyaline rim laterally. The proliferating cartilage appeared to have a hyaline matrix with subsequent merging with fibrocartilage. The degree of peripheral reactivity was greater than the controls and progressed with healing time. The drill hole could be seen in the sections with evidence of progressive filling of the surface defect with granulation tissue, fibrous tissue, and subsequently fibrocartilage as healing time advanced from one to 3 weeks.

FOUR MM DEFECTS

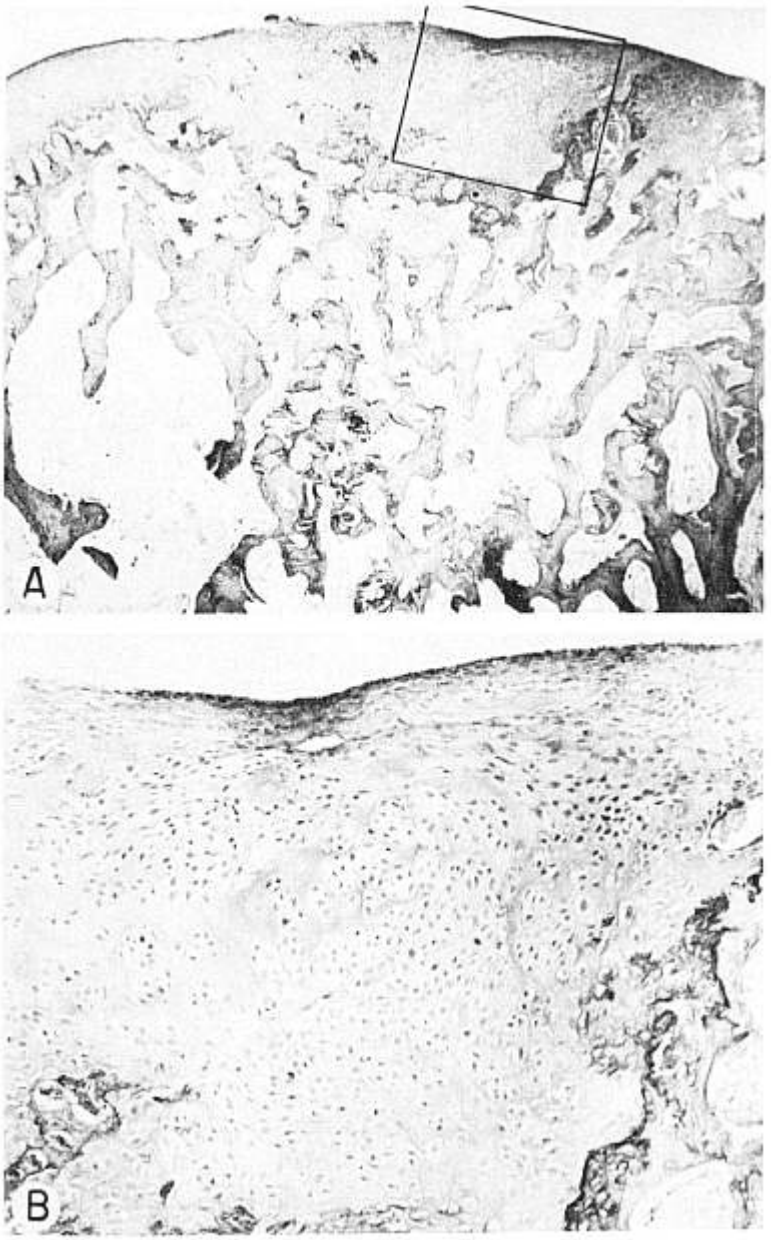
Controls. The gross appearance of the healing defects revealed rounding of the marginal rims of the defect, central yellow-white soft tissue with obliteration of the drill holes



FIGS. 6A and B. (A) Specimen at two weeks after insertion of a 10 megohm device in a large defect reveals an island of cartilage with a focus of hyaline cartilage in a predominately fibrous matrix. (×30) 90% (B) Inset showing a hyaline cartilage repair response in a field of fibrous tissue. (×100)

and one specimen with whitish cartilaginous appearing material encroaching upon the defect from the peripheral rim. Light microscopy demonstrated frequent peripheral cellular reactivity of one plus degree with rounding and thickening of the marginal rim. The central portion of the defect displayed fibrous tissue covering the subchondral bone with occasional evidence of fibrocartilage (Fig. 10). One specimen demonstrated significant change with one mm of peripheral encroachment by hyaline cartilage, two plus marginal proliferation of chondrocytes, two plus central reactivity at the site of the drill hole and increased thickness of the cartilage at the margin of the defect as compared with the unoperated medial condyle (Fig. 11). The encroaching cartilage appeared to be growing over cancellous bone that was covered with fibrous tissue. The impression gained while examining the serial sections was that proliferation of the marginal cartilage was occurring with tongues of cartilage growing centripetally over the defect. There also appeared to be fibrous and fibrocartilaginous repair extending from the subchondral bone.

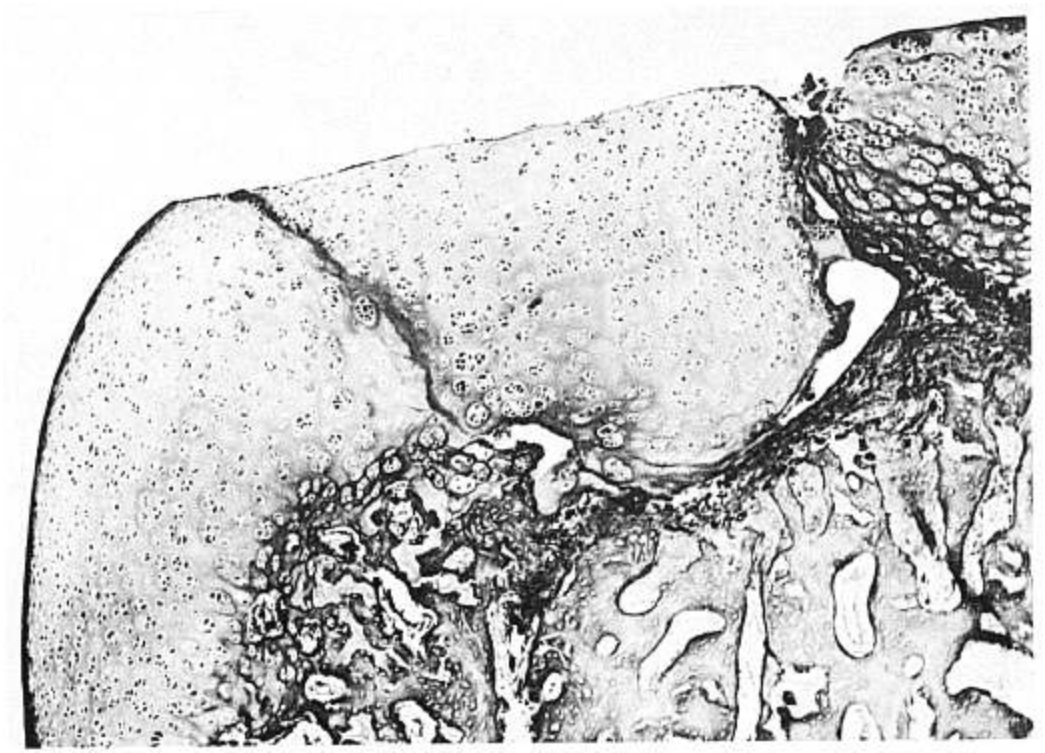
Epoxy. Evaluation of the devices revealed no problems with breakage as was observed with the previous group of epoxy devices.

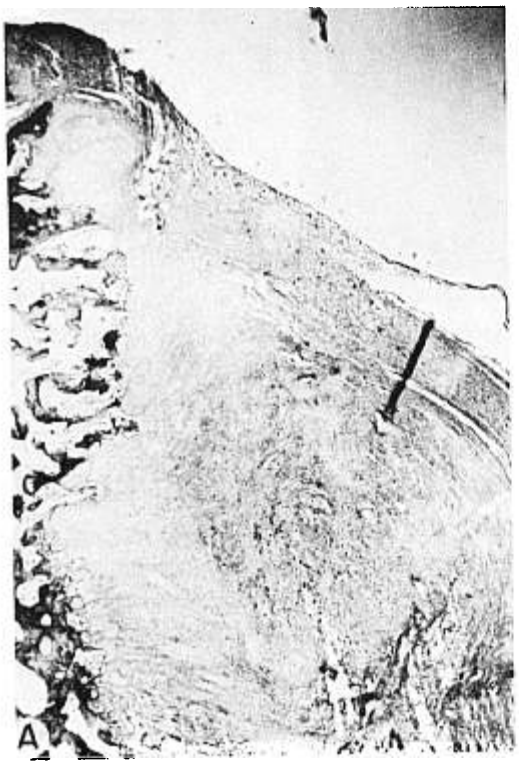


FIGS. 7A and B. (A) 10 megohm device in a large defect at 14 days revealing central proliferation of a focus of fibrocartilage in a field of fibrous tissue with apparent origin from the subchondral elements. (×30) (B) Inset demonstrating the focus of fibrocartilaginous proliferation. (×100)

FIG. 8. (top) A thickened margin of cartilage repair with evidence of cellular proliferation and thickening of the articular cartilage at the margin of a 4 mm control defect at 21 days. (×54)

FIGS. 9A and B. (A) (bottom left) Control specimen with a large defect at three weeks showing marginal proliferation of cartilage with a blending of the cartilage into a fibrocartilaginous matrix. (×90) (B) (bottom right) specimen with a large defect and a 10 megohm device at 3 weeks demonstrating marginal proliferation of cartilage to a greater degree with subsequent blending into a fibrocartilaginous matrix. (×100)

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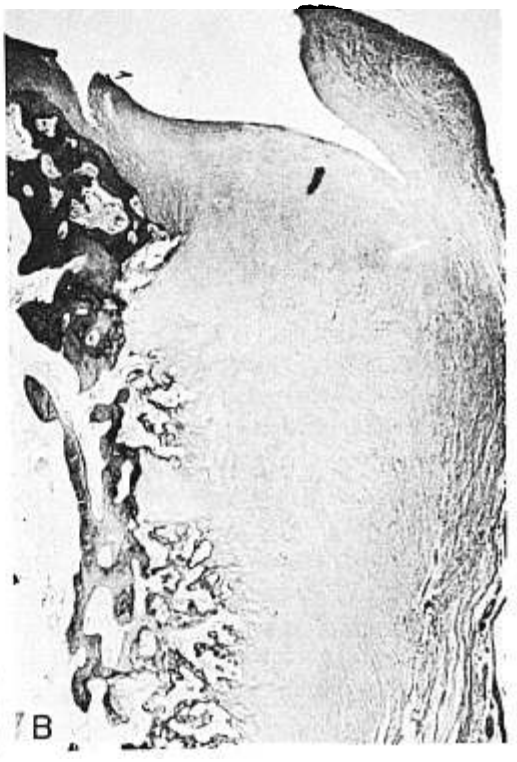
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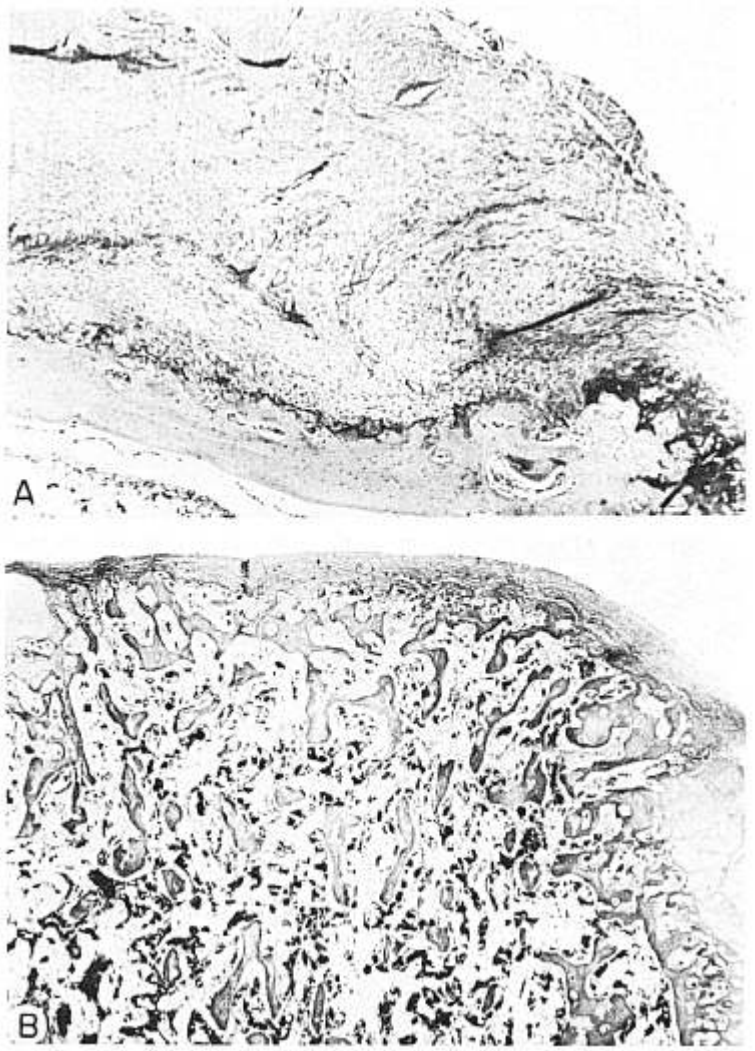
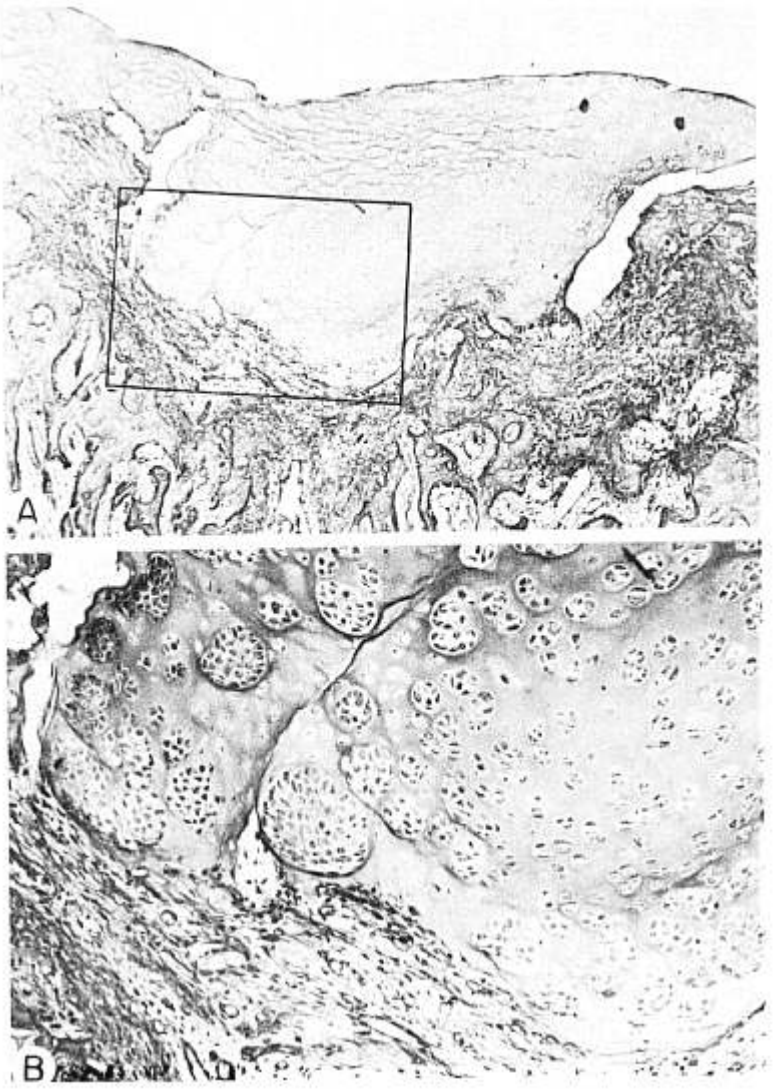


FIGS. 10A and B. (A) Fibrous response in a control animal with a four mm defect 62 days after surgery. (×22.75) (B) Fibrous response two weeks after creation of the surgical defect with evidence of the fibrous tissue covering the subchondral bone and little marginal response. (×15)

There was no evidence of infection. Grossly the defects showed rounding of the margins of the defect with yellowish-white central soft tissue. The drill holes were not apparent. One specimen had one mm of encroachment from the superior margin of the original defect. There was 2+ marginal proliferation and 1+ islands of cartilage, 1+ central reactivity and increased thickness of the articular cartilage at the margin.

Ten Megohm. Evaluation of the devices revealed no breakage, no abnormal reactive tissue, and no evidence of infection. Grossly there was evidence of rounding of the marginal rims and encroachment on the defect in 71 per cent of the condylar surfaces. There was evidence of some central yellow-white soft tissue reaction. The drill hole was obliterated on the surface. Microscopically, 69 per cent of the specimens demonstrated 0.5 to 1.0 mm of marginal encroachment, 92 per cent showed marginal proliferation, while 38 per cent showed islands of cartilage with no apparent connection to the marginal car-



FIGS. 11A and B. (A) Control specimen with a 4 mm defect at 3 weeks demonstrating a hyaline cartilage repair response with thickening, cellular proliferation and evidence of matrix production. (×40) (B) Inset from (A) evidence of a fibrous base over subchondral bone with cellular proliferation and chrondron formation in the repair response. (×100)

tilaginous rim. Thirty-one per cent showed marked cellular proliferation centrally with fibrous tissue and fibrocartilage, occasionally covered by hyaline cartilage. Fifty-four per cent showed increased thickness of the original marginal rim as compared to the unoperated medial condyle. Table 1 represents tabulation of the grading of each category of tissue response and total score for each specimen. A scanning electron micrograph of one specimen revealed rounding of the marginal rims with apparent proliferation of the smooth surface tissue extending from the margin (Fig. 12). Tongue-like projections extended as far as one mm centrally appearing to "leap-frog" the healing tissue beneath. The surface of these tongues was smooth except for some evidence of pitting while the central portion of the defect was covered with a more rough appearing tissue.

Sections stained with toluidine blue showed evidence of metachromasia and a histological

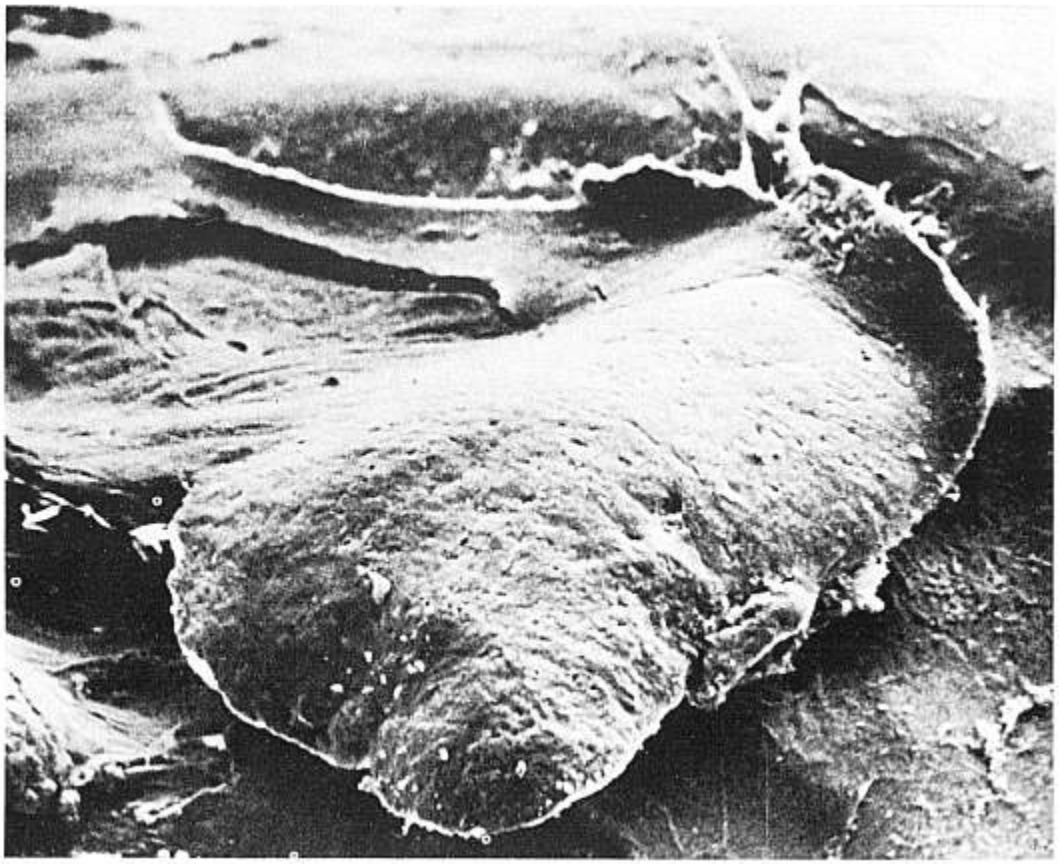


FIG. 12. A scanning electron micrograph of a tongue of cartilage extending from the rounded articular margin of a 4 mm defect 21 days after surgery with a 10 megohm device inserted. (×75)

picture of repair tissue compatible with hyaline cartilage (Fig. 13).

Transmission electron micrographs of the encroaching repair tissue were similar in character to those described by Ghadially.[17] The matrix had a mottled appearance with electron dense bodies and various sized collagen fibers. The chondrocytes had few cell processes and there were large amounts of rough endoplasmic reticulum (Fig. 14).

RESULTS

The impression gained after reviewing and grading the specimens, grossly and microscopically, was that 3+ or greater in total grade represented a significant response (Table 2). In the large defects, only one specimen, representing 11 per cent of the 10 megohm specimens demonstrated a significant response. A significant response was seen in the 4 mm defects in 14 per cent of the controls, 50 per cent of the epoxy and 8 of 13, or 62 per cent of the 10 megohm specimens.

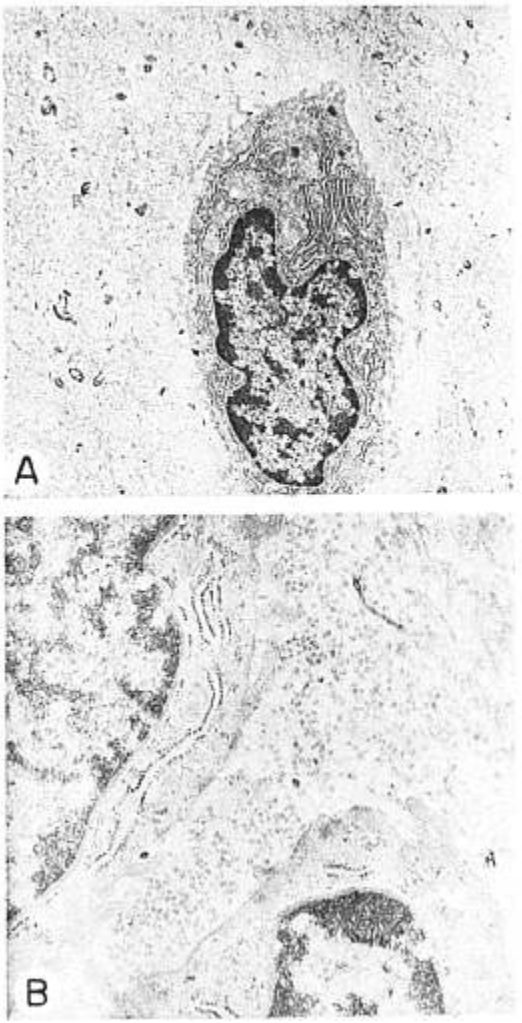
DISCUSSION

Our studies support the concept that a potential for regrowing hyaline cartilage at the site of a defect does exist and that this potential can be enhanced by changes in the electrochemical environment. The sources of

the repair tissue appear to be from two areas. Subchondral marrow elements appear to supply mesenchymal cells to heal the raw bony surface with fibrous tissue. This appears to convert to fibrocartilage. Some of our results show hyaline cartilage covering fibrous tissue and fibrocartilage at the center of the defect. This is consistent with the findings of Shands[30] and others[15, 29] who support the concept of repair through metaplasia. If this source of tissue repair was supplying the major portion of the cartilage, however, one would expect a more dramatic response in models with large articular defects. This was not so and the most significant response in these defects was the appearance of hypercellularity and thickening of the remaining rim of hyaline cartilage over the lateral nonweight bearing region of the condyle.

The hypercellularity and encroachment of the marginal articular cartilage as seen in the models with 4 mm articular defects supports the findings of other investigators.[10, 12] Giant chondron formation, with 30 or more cells clumped together, was seen with small rims of hyaline-appearing matrix about each cell. There was evidence of mitotic activity in the cells of the chondron. Carlson[12] has shown that these cells are metabolically active and have an increased uptake of S^{35} indicating increased production of chondroitin sulfate.

The gross appearance of the 4 mm defects in scanning electron micrographs demonstrated rounding of the margins and the appearance of tongue-like projections of repair tissue growing centripetally from the margin, corroborating the image created from reviewing the serial sections with light microcropy. Histologically, these tongue-like projections had a hyaline matrix when stained with toluidine blue and with hemotoxylin and eosin. Transmission electron micrographs show cells and matrix compatible with normal hyaline cartilage. The cartilage appeared to grow over fibrous repair tissue and fibrocartilage covering the raw, bony surface with consistent evidence of connection to the margin. The fact that exuberant reaction was seen in one control animal suggests that a major poten-



FIGS. 13A and B. (A) Transmission electron micrograph demonstrating the repair response at 28 days with a 4 mm defect and a 10 megohm device inserted. The matrix appears mottled and there are various sized fibers while in the cytoplasm there are moderate amounts of endoplasmic reticulum. The findings are compatible with the appearance of normal hyaline cartilage. (×6,970) (B) A similar area at ×17,000.

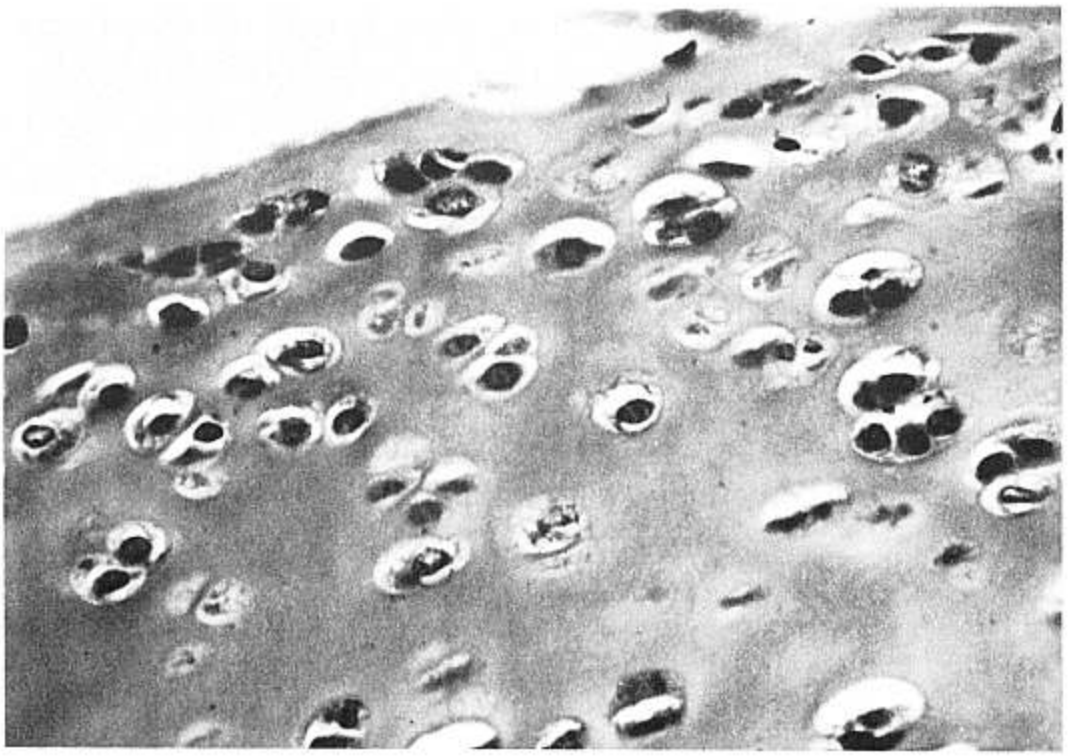


FIG. 14. A 20 day specimen with a 4 mm defect and 10 megohm device inserted. The specimen has been stained with toluidine blue and there is evidence of metachromasia and a homogeneous matrix on the original color slides. (×213.60)

tial for spontaneous proliferation of hyaline cartilage does exist in a small percentage of cases. Campbell[11] has stated that occasional hyaline repair is seen but that this occurs infrequently even among animals of the same species. The result of the models with four mm defects in which bi-metallic devices were used showed a reaction of three plus or greater in 62 per cent of those with a 10 megohm device implanted and 50 per cent of those with an epoxy device implanted while a 3+ or greater response was seen in only 14 per cent of the controls (Table 2). This suggests that significant enhancement of the animal's natural capability to repair articular cartilage can be achieved by appropriate changes in the electro-chemical environment.

Further studies dealing with the type of devices, the optimum levels of voltage and current, and the exact means by which the cells are stimulated are needed. Also, the effect of similar changes in the electrochemical environment of articular cartilage defects in mature animals remains to be investigated.

SUMMARY

Full thickness defects in mammalian articular cartilage heal with fibrocartilaginous tissue. Articular cartilage subjected to chronic trauma or osteoarthritic changes responds by chondrocyte proliferation and increased biochemical activity. Immature rabbit articular cartilage is capable of limited cellular proliferation in response to surgically created full thickness defects.

Light microscopy, transmission electron microscopy and scanning electron microscopy were used to demonstrate enhancement of this latent healing potential with changes in the electrochemical environment produced by bimetallic electrochemical devices.

TABLE 1. Repair Scores for Condyles In Each Scoring Category With Regard to Post-Implantation Time, Device Used and Defect Type

		Large Defects			
Time		*1 Week*	*2 Weeks*	*3 Weeks*	
Device	*Category**				
Ten Megohm	A	0 0 0	0 0 0	0 0 0	
	B	1 1 0	1 1 1	0 1 1	
	C	0 0 0	0 0 0	0 1 0	
	D	0 0 0	1 0 1	1 0 0	
	E	0 0 0	0 0 0	0 1 0	
Total Scores		1 1 0	2 1 2	1 3 1	1.33 Mean
Epoxy	A	0	0 0 0	0 0	
	B	0	0 1 1	1 1	
	C	0	0 0 0	0 0	
	D	0	0 0 1	1 1	
	E	0	0 0 0	0 0	
Total Scores		0	0 1 2	2 2	1.17 Mean
Control	A		0 0	0 0 0	
	B		1 0	1 0 1	
	C		0 0	0 0 0	
	D		0 0	1 0 0	
	E		0 0	0 0 0	
Total Scores			1 0	2 0 1	0.8 Mean

		4 mm Defects				
Time		*1 Week*	*2 Weeks*	*3 Weeks*	*9 Weeks*	
Device	*Category*					
Ten Megohm	A	1 0	1 0 1 1 1 0	1 1 2	2 0	
	B	2 1	1 1 1 0 2 1	1 1 1	2 1	
	C	1 1	0 1 0 1 0 0	0 2 0	0 0	
	D	0 0	0 0 0 0 0 1	0 2 0	2 1	
	E	0 0	1 0 0 0 1 1	2 1 1	2 0	
Total Scores		4 2	3 2 2 2 4 3	4 7 4	8 2	3.62 Mean
Epoxy	A	0 0	1	2		
	B	1 1	2	2		
	C	1 0	0	1		
	D	0 0	0	1		
	E	0 0	0	2		
Total Scores		2 1	3	8		3.50 Mean
Controls	A	0 0	1	0 2	0	
	B	1 1	1	1 2	2	
	C	0 1	0	0 0	0	
	D	0 0	0	0 2	0	
	E	0 0	0	1 2	0	
Total Scores		1 2	2	2 8	2	2.71 Mean

* Category A: Extent of marginal encroachment of hyaline cartilage over the defect site.
Category B: Degree of marginal chondrocyte proliferative activity.
Category C: Degree and frequency of islands of hyaline cartilage appearing within the defect site.
Category D: Degree of cellular proliferation in the central defect.
Category E: The degree of increased thickness of marginal articular cartilage.

TABLE 2. Total Repair Scores Expressed as Per Cent of Specimens With Each Implant Type and Defect Size

			Large Defects		
*Total Scores** *Device*	*1*	*2*	*3*	*4*	*No. of Condyles*
Ten Megohm	89%	33%	11%	0	9
Epoxy	67%	50%	0	0	6
Control	60%	20%	0	0	5
			4 mm Defects		
Total Scores *Device*	*1*	*2*	*3*	*4*	*No. of Condyles*
Ten Megohm	100%	100%	62%	46%	13
Epoxy	100%	75%	50%	25%	4
Control	100%	86%	14%	14%	7

* Total scores of condyles represented as percentage of specimens with that score or greater, for each implant type and defect size.

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