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Chondrocyte Allograft Transplantation for Damaged Growth Plate Reconstruction

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The growth plate is responsible for longitudinal bone growth. The problem of repair of damaged growth plate in children has never been adequately solved. The purpose of this study is to investigate the ability of the cultured chondrocyte for the prevention of bony bridge and repairment of damaged growth plate. Chondrocytes were obtained from the new born canine epiphyseal plate and was cultured in high density.

Fourteen days later they formed micromass easily removable from the culture flask. Twenty dogs were divided into two groups; in group I, the medial proximal tibial growth plate was destroyed and then cultured chondrocytes were transplanted into the defect, and in group II, the medial proximal tibial growth plate was left in destroyed state. Each left leg was remained as a control. The growth pattern was observed radiographically and histologically until 16 weeks after graft. 4 weeks after the operation, the angular deformity had been observed, and 31° of angulation was noted at the 16th week in group II, while there was less than 8° of angulation and nearly normal growth in most of dogs of group I (8 of 10 dogs). The other 2 dogs had shown 20° angulation. In group II, there was definite bony bridge on the medical proximal growth plate. In group I, initially, the cultured chondrocyte remained as a amorphous cartilagenous mass, but as time progressed, amorphous cartilagenous mass had formed cartilagenous matrix which was proved by Safranin-O staining. Although this study showed the role of cultured chondrocyte as a method of preventation of bony bridge formation and possibility to repair of growth plate, further studies should be done to prove the reconstruction of the growth plate.

Key Words: Chondrocyte transplantation, culture, growth plate

Longitudinal fracture through growth-plate elicites bony bridge which was developed by crossing from the epiphysis to the metaphysis. Such a bony bridge may produce an effect of epiphysiodesis and subsequent localized growth arrest. Clinically the growth plate or physis is involved in 6~15% of children's fracture and is responsible for longitudinal bony growth. Of these injuries, 25~35% have been reported to result in some shortening or deformities, but in only 10% are the deformities lead severe functional problems(Rogers 1970). Many of these deformities tend to involve either the distal femoral or the proximal tibial plate, leading to valgus or varus deformites. A late sequele of these deformities may be degenerative arthritis.

Experimental and clinical attempts to abolish the arrest of in vivo bony growth include interpositional physiolysis with biologic and prosthetic materials, electric current stimulation, transphyseal and diaphyseal distraction, and physeal transplants. Yet, they have some

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limitations(Hass 1919; Phemister 1933; Ring 1955; Freeman 1959; Harris *et al.* 1965; Eades and Peacock 1966; Laurence and Smith 1968; Green 1971; Osterman 1972; Hoffman *et al.* 1972; Becker *et al.* 1977; Langenskiöld 1981; Olin *et al.* 1984; Foster *et al.* 1984; Foster *et al.* 1986). The purpose of this study is to investigate the ability of the cultured chondrocyte to prevent bony bridge and repair of damaged growth plate.

MATERIALS AND METHODS

Grafting Procedure

Growth plate cartilage was obtained from the shoulder, iliac crest, and knees of 5 day-old Korean dog, weighing 750 g. The whole scapula, iliac crest, hip, elbow, and knees were removed immediately after the animal was killed by euthanasia. Then the removed materials were immersed in Gey's balanced salt solution(GBSS, PH; 7.0, Gibco, USA) added with 100 μ/ml penicillin G, 100μg/ml streptomycin, and 1 μg/ml amphotericin B, then dissected under sterile conditions in a laminar flow hood.

After the identification of the epiphyseal plate, the periosteum was carefully dissected away, and the perichondrial growth ring was excised. The plate was divided through the zone of provisional calcification, and was seperated from the ossification region of the epiphysis with a spatula. All bony tissue was removed from both sides of the plate. The tissue was diced into 1~2mm^3 segments in GBSS and digested sequentially with 0.5% hyaluronidase (Sigma) for 15 minutes, 0.2% trypsin (Sigma type IX) for 30 minutes, and 0.2% collagenase (Sigma) for 125 minutes. The enzymes were prepared in sterile GBSS(Gibco) in PH 7.0; the digestion was done in conical tube at 37℃. During each step of digestion, the specimen was washed frequently with GBSS for purification.

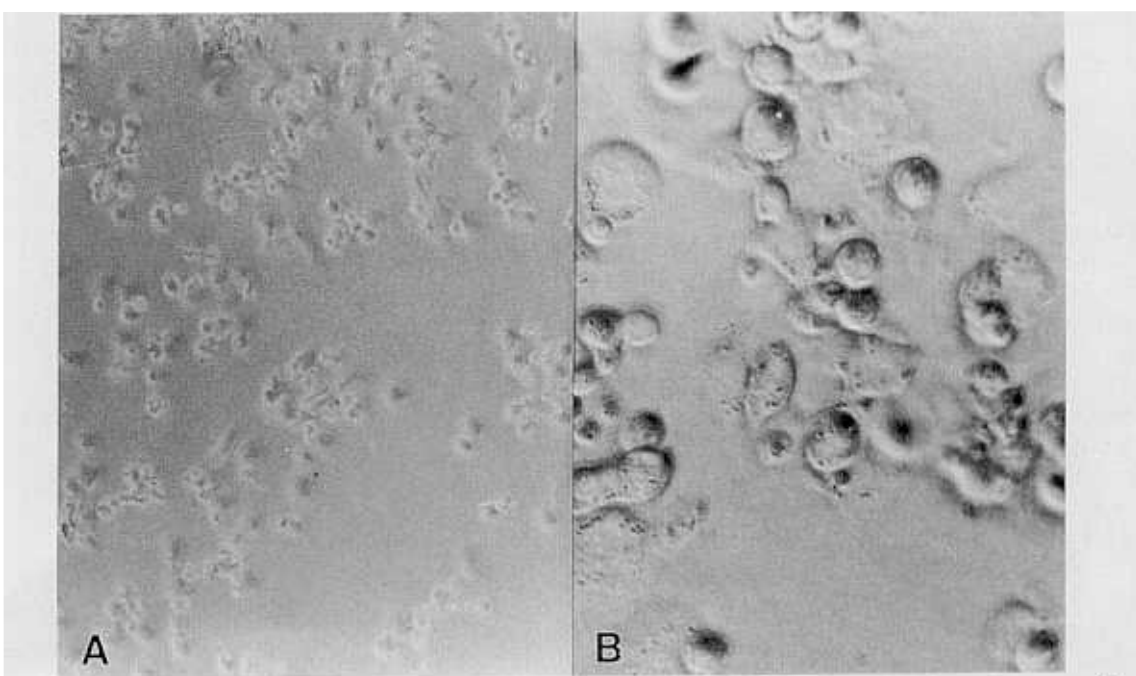

Fig. 1-A, B. Light biomicroscopic finding of cultured chondrocytes. Clusters of chondrocytes and matrix surrounding each cells are shown which was characteristic of chondrocytes (1-A; ×100 1-B; ×400).

After digestion, specimens were centrifuged at 600G for 8 minutes in order to pellet the cells. The supernatant was discarded and sedimented materials were resuspended in F-12 medium with 20% Fetal Calf serum and recentrifuged at 180G for 1 minute to sediment undigested martrix particles. The F-12 medium was added with 12% Fetal Calf serum, 2.3 mM Mg^{2+}, 100U/ml penicillin G, 100 g/ml streptomycin SO_4, PH 7.6. The supernatant was recentrifuged at 600G for 100 minutes to sendiment the cells. And the sedimented cells were resuspended in F-12 medium with 12% Fetal Calf serum. Cell counts were done in a hemocytometer and added with 0.5 ml of 0.1% trpan blue to 0.5 ml of incubated cells for determmiation of viability. Cells were innoculated into 12 cm×2 cm plastic culture wells at a density of 10^7 viable cells/cm^2 and cultured in a atmosphere of 95% air and 5% CO^2 at 37℃ The nutrient medium was changed every day.

By the 14th day, the cells formed a cohesive disc then easily removed from the culture well with forceps, and were easily transplanted to growth plate defects(Fig. 1).

Experimental model of growth plate injury & grafting procedures

The model of hemiepiphyseal arrest by the technique of Phemister(Phemister 1930) has been previously described. Five day-old dogs were used. Postoperatively, radigraphs were taken at the time of surgery and every week in the anteroposterior and lateral projection until the animals were killed. The legs of dogs were always held in a special holder so that the legs of dogs remained in the ventral position. A medial approach was made on the proximal tibia; by extraperiosteal dissection, the medial aspect of the proximal tibia & its growth plate were identified. A block of 10 mm length, 5 mm width, and 3 mm depth was removed, being 7 mm distal and 3 mm proximal to the plate, and reversed as in Phemister's description(Phemister 1933). A cut in the bone was made with a scalpel, and the block was elevated, removed, and cultured chondrocyte was implanted into these defect of 10 dogs.

Each animal served as its own control, because only the right knee was operated, therefore the growth of the unoperated, opposite, normal side could be determined.

Another controls included 10 dogs in which the epiphysiodesis was not disturbed. In those animals, growth on the epiphyseal defect side was compared with the growth on the unoperated side. Animals were killed immediately after operation, 2, 4, 6, 8, and 16 weeks after transplantation.

Radiological Evaluation

Both shortening and deformity were measured. Tibial length was determined by measurements from the intercondylar notch of the proximal tibia to the distal tibial articular surface. Shortening represented the difference

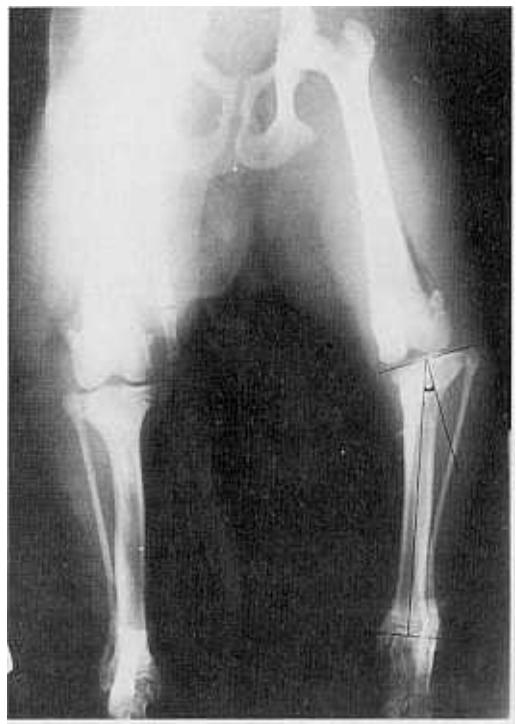

Fig. 2. *The technique used in measurements of the deformity is demonstrated. It is represented by the angle fomed by the lines perpendicular to the distal femoral and distal tibial articular surfaces.*

between the operated tibia and the opposite control tibia. The tilt of the tibial plateau produced by the hemiepiphysiodesis remained parallel to the articular surface of the femoral condyles.

The degree of angular deformity of the tibia was determined by measuring the angle formed by the lines perpendicular to the distal femoral articular surface and to the distal tibial articular surface then by calculating the difference between the degree of angular deformity immediately after transplantation and at harvest time(Fig. 2). In all animals, a 26 gauge needle was inserted through the width of proximal tibia as a marker for specific determination of whether the growth was occuring in the transplanted cells. If the plate was growing normally on the transplanted side, the distance from the plate to the marker would increase.

Histologic Studies

Cultured chondrocytes were stained with safranin-O and toluidine blue for detection of glycosaminoglycan. Specimens from dogs were fixed in neutral formalin, decalcified in EDTA, and embedded in paraffin. Sections, 6μm thickness, were cut through the grafted area and stained with Safranin-O fast green, toluidine blue, hematoxylin and eosin, and alcian blue along with their counterstains so that the presence and distribution of glycosaminoglycans were shown.

RESULT

Radiological changes

In the epiphyseal defect group, the knees that had received epiphyseal defect operation were gradually bowed and shortened(Fig. 3). After operation, the shortening and varus deformity were progressively increased. The angular deformity had been observed since 4 weeks after the operation, and finally 31° angulation was noted. And osteoarthritic change was seen in advanced cases. But those animals with live cell transplantation did not develop the same deformity as those with epiphyseal defect group(Fig. 4).

Eight of ten animals showed changes of less 3° in the deformity factor eight weeks after transplantation, the other two animals showed an increase in deformity by an average of 12.7°.

At final follow up after transplantation, eight specimens showed more progressed deformity factor as compared to that of the eighth week; the final deformity factor was 8° in average. Other two specimens showed 20° of deformity factor and a shortening of 1.3 cm.

Gross and Histologic Findings

On final gross findings of specimen , the chondrocyte transplanted group showed minimal angular deformity on the proximal tibial plateau as compared to normal right side, and the chondrocyte mass was shown well incorporated into the surrounding growth plate(Fig. 5). In contrast, the epiphyseal defect group showed marked angular deformity on the proximal tibia inclined onto the defect side and with shortening of tibial length. Osteoarthritic changes were also shown. And at close up finding the definite bony bridges were noted(Fig. 6). Two weeks after transplantation, the grafted cells showed somewhat amourphous cartilagenous mass around the defect area(Fig. 7-A, B).

Four weeks after grafting, the grafted cells had made contact with the surrounding bone. And these cells had progressed to somewhat columnar arrangement as in the adjacent normal growth plate which might suggest the endochondral ossification process like the normal growth plate(Fig. 7-C). Some lymphocytes were seen in the area of the transplanted cells, possibly representing an immunologic reaction(Fig. 7-C).

Eight weeks after transplantation, the transplanted matrix still had metachcomatic staining. Enchondral ossification was proceeding with what looked like a growth plate(Fig. 7-C). And these findings continued until specimens of 16th week. In contrast, the animals that had epiphyseal defect showed well formed bony bridge across the growth plate (Fig. 8).

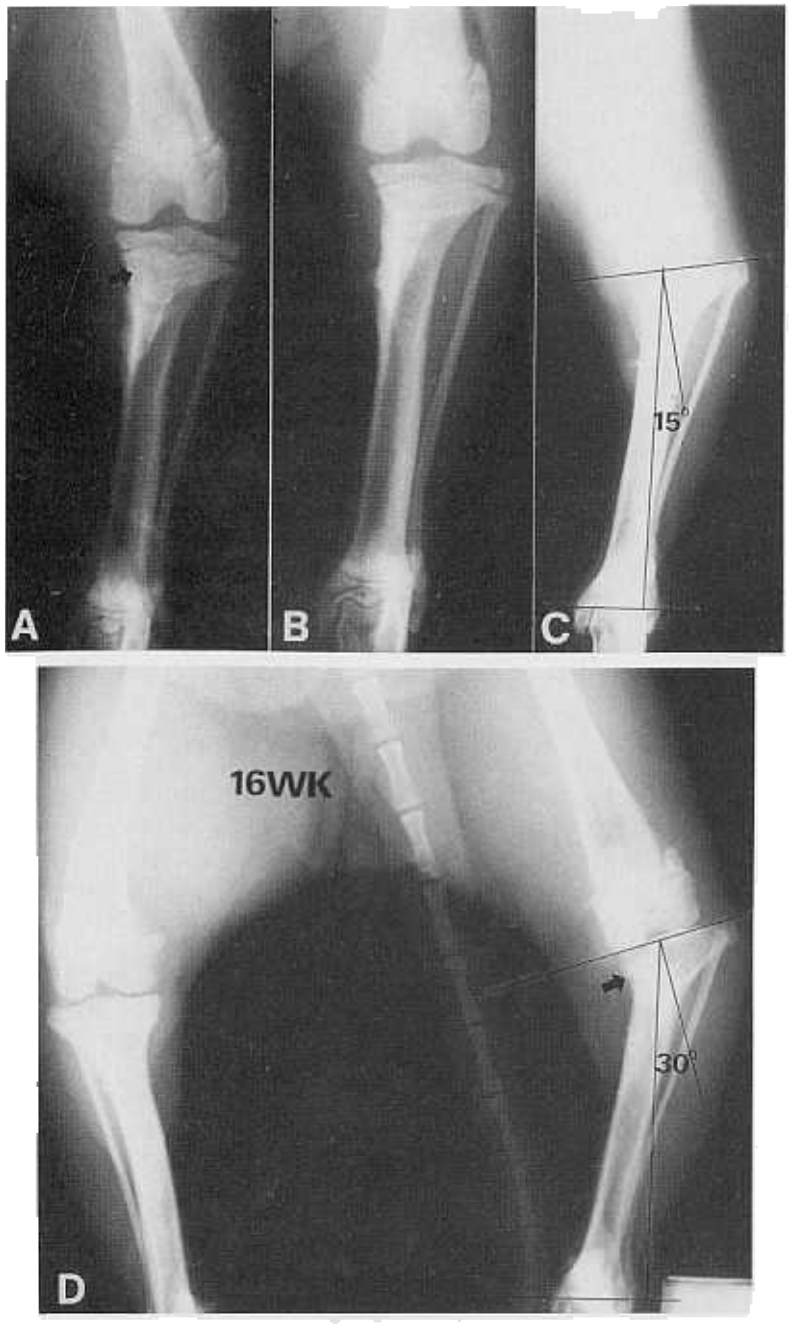

Fig. 3A-D. *Serial radiographic changes after epiphyseal defect formation on the medial aspect of left proximal tibia.*

A. Radiograph taken immediately after epiphyseal defect formation(arrow).

B. Four weeks after operation there was noted slight but not remarkable angular deformity.

C. Eight weeks after operation angulation was visible and the deformity factor was 15 degrees.

D. Sixteen weeks after operation marked angular deformity of which deformity factor was 30 degrees was noted on the defect area also degenerative change was visible on the involved joint(arrow.)

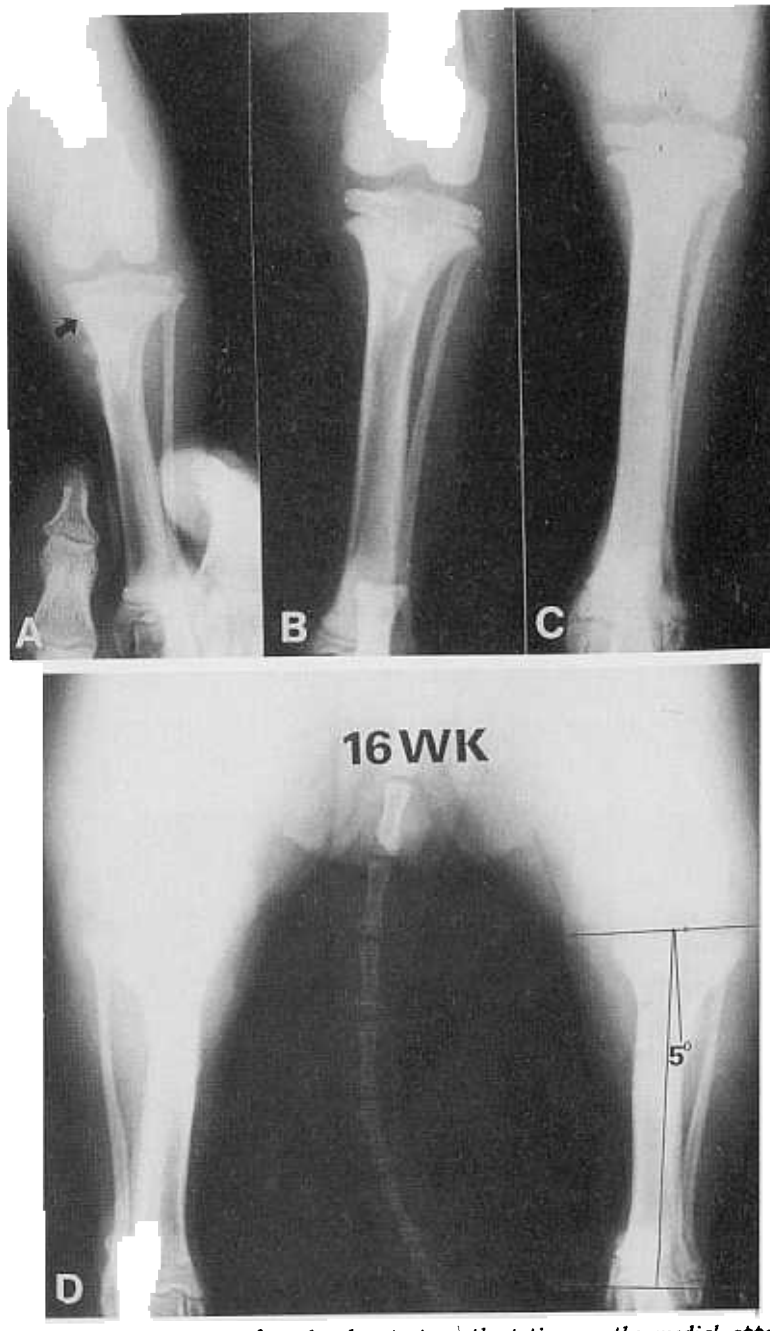

Fig. 4A-D. *Serial radiographic changes after chondrocyte transplantation on the medial appect of proximal tibial epiphyseal defect area.*

A. Radiographs taken immediately after chondrocyte transplantation (arrow).

B. Four weeks after transplantation there was no remarkable change on the transplanted area compared to epiphyseal defect group of Fig. 3-B.

C. Eight weeks after transplantation. No angular deformity was noted compared to epiphyseal defect group of Fig. 3-C.

D. Sixteen weeks after transplantation. The deformity factor was only 5° on the left tibia.

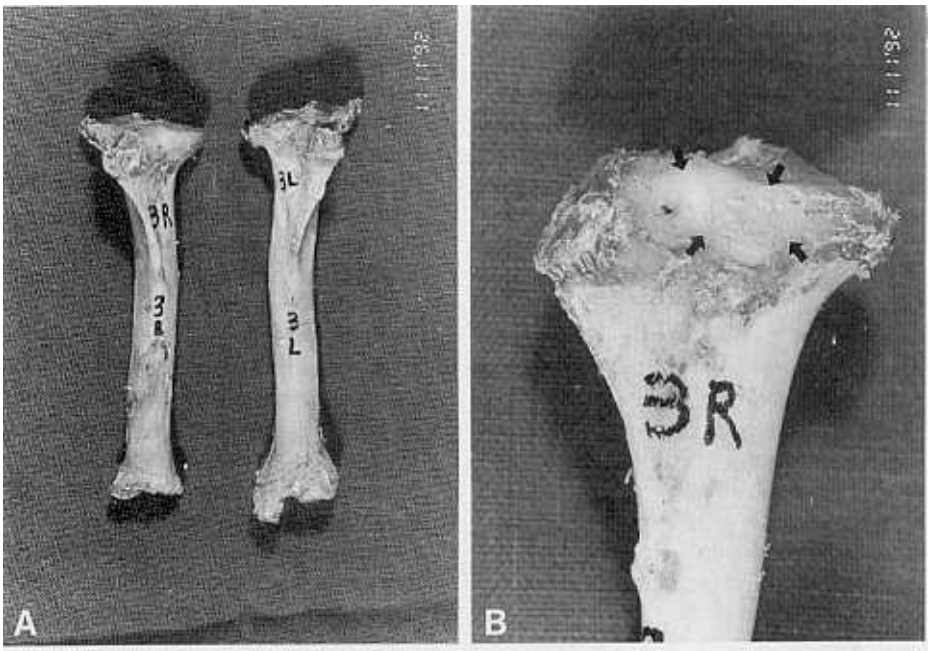

Fig. 5-A, B. *Gross photographs of canine tibiae in chondrocyte transplanted group.*
5-A. *There was little angular deformity.*
5-B. *The chondrocyte mass was shown as well incorporated into surrounding growth plate cartilage(arrows).*

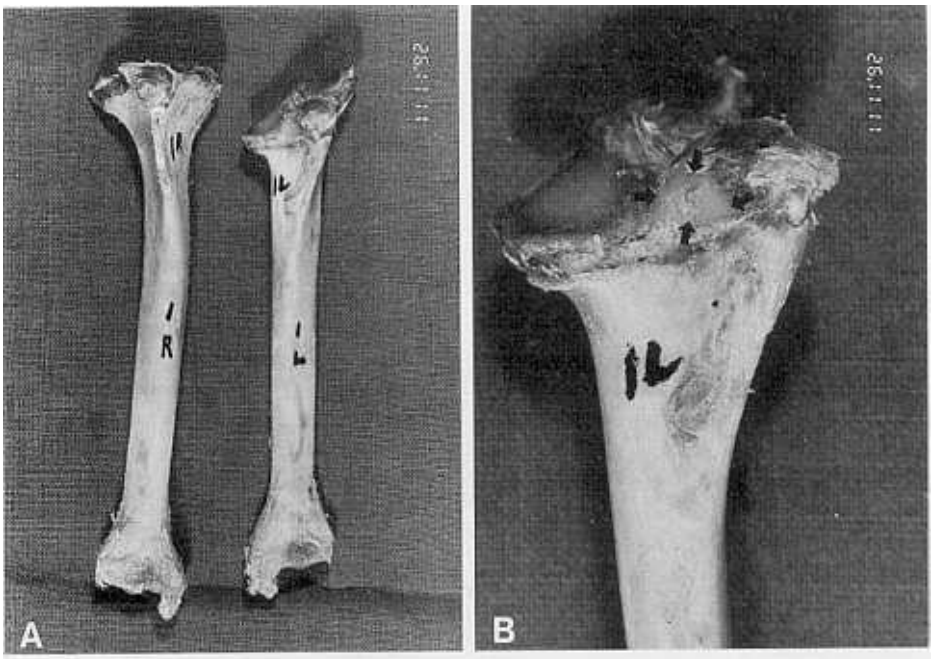

Fig. 6-A B. *Photographs of canine tibiae after epiphyseal defect procedure.*
6-A. *The left proximal tibia showed marked angular deformity and shortening.*
6-B. *On close up finding bony bridge was shown(arrows).*

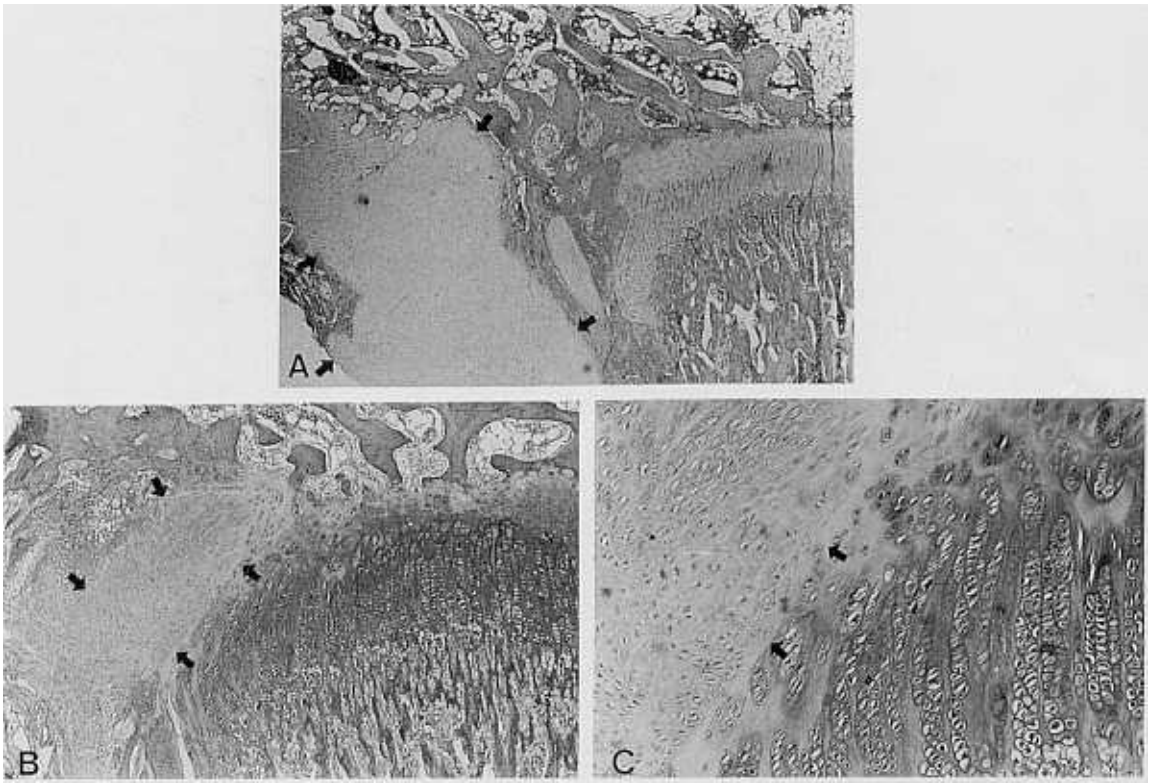

Fig. 7-A, B, C. *Photomicrographs of chondrocyte transplanted growth plate.*

7-A, B. *4 weeks after transplantation the chondrocyte forms a amorphous mass around the defect area (Black arrows in 7-A B). White arrows indicate adjacent normal growth plate with columnar arrangement of cells characteristic of endochondral ossification process.*

7-C. *Eight weeks after transplantation. This amorphous mass has progressed to somewhat columnar arrangement which might suggest endochondral ossification (Arrows in 7-C). (Stain: safranin-O fast green, ×10 in A, ×20 in B, ×40 in C).*

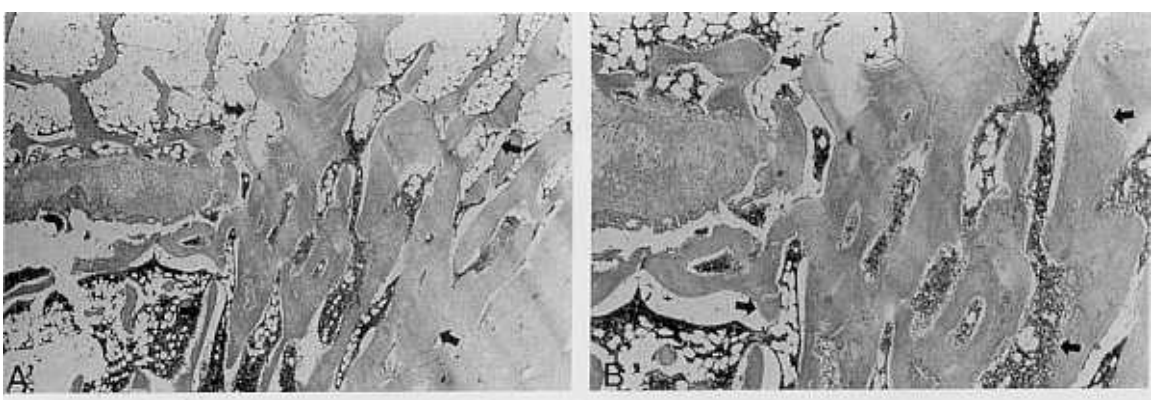

Fig. 8-A, B. *Photomicrographs of epiphyseal defect canine tibia group. On the medial side of the proximal tibia, well formed bony bridge across the cartilagenous growth plate was seen (arrow in 8-A, B, Safranin-O-fast green, × 10 in A, × 40 in B).*

DISCUSSION

There have not been any definite solving tool for the repair of growth-plate injury and its subsequent sequela such as bony bridge that causes deformity of shortening or angulation of long bones. Although work on the use of inert fillers in growth plate defects has been extensive, these fillers have served solely to prevent bony bridges from reforming. Therefore, repair of growth plate defect depends on the remaining cells in the plate. In fact, Bright(Bright 1974, Bright 1981) has demonstrated that normal growth cannot continue if more than 30% of the plate has been lost. The current notion is that prevention of the formation of bony bridges across the cartilagenous growth plate will prevent growth arrest. Indeed, early observation by Osterman (Osterman 1972) of the arrest of bony bridge formation by implanted deep-frozon hyaline cartilage is of particular interest.

A satisfactory orthopaedic outcome of introducing a cartilage transplant into growth-plate defect must recognize at least two provisions; (1) the prevention of bone-bridge formation, and (2) the restoration of orderly growth plate functions in growth and development of the limb.

Launence and Smith(Laurence and Smith 1968) presented evidence for a slowing of union in experimental bone defects into which freshly isolated articular cartilage cells had been implanted. Exploitation of this characteristic by chondrocyte implants to prevent bone-bridge formation would thus satisfy the first provision. In this series, the cultured chondrocyte showed that they prevented bony bridge formation both in radiological findings and more definitely in histologic finding, so that cultured chondrocytes satisfied at least one of the suggestions mentioned previously. In this study, the fact that the cells remained alive, as implied by the continuous maintenance of the safranin-O-stained matrix, suggested that the graft retained its potential for growth. The most striking question to be asked here is why the cells in the transplanted plate were not immunologically rejected. There has been controversy concerning the fact that cartilage seems to be a tissue relatively protected from immunologic attack. Many studies showed that the matrix surounding chondrocyte plays a role as a immunologic barrier so that the antigenicity of chondrocyte was not exposed to circulating immunologic system(Freeman 1959; Bently and Greer 1971).

Amadio *et al.*(Amadio *et al.* 1983) recently reported that high-density primary cultures of bovine epiphyseal chondrocyte synthesize aggregatable proteoglycan and type II collagenat up to 15 days of the same type found in vivo plate, so that this similarity of the matrix may be the cause of immunologic escape. Many authors suggested that high density cultures may be the key factor in synthesis of chondroid matrix, which presumably protected them. These expereiment suggest that allografts of cultured epiphyseal cells can survive, produce matrix, and become incorporated into subchondral bone in the tibial epiphysis of the young dog.

But this is a pilot study, and a larger number of aminals should be studied before this result is applied to clinical trials. And the limitation of this study is caused by absence of autoradiographic study which is the clue of survival of chondrocyte allograft. Futhermore, we have not studied the immonologic reaction of the allograftcells. Therefore, further studies are necessary in order to solve these problems and utilize the result of this study, clinically.

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