Treatment of growth plate injury with autogenous chondrocytes

A study in rabbits

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ABSTRACT – We designed this study to investigate transplantation of autogenous chondrocytes cultured in atelocollagen gel to treat the injured growth plate. An experimental model of growth arrest was made by resecting the medial two thirds of the left proximal tibial physis in 8–10-week-old Japanese white rabbits. Autogenous chondrocytes, which had been harvested from cartilage of the knee joints, embedded in atelocollagen gel, and cultured for a week, were transplanted into the defect in the growth plate. In two other experimental groups, we transplanted autogenous fat tissue into the same defects, or left them empty.

Histological and radiographic examinations were done before and after transplantation at various times up to 52 weeks postoperatively. The histological study showed that grafted chondrocytes synthesized extracellular matrix and prevented early ossification and closure of the growth plate, which occurred in the Fat and Defect groups. Angular deformity and length discrepancy in the transplanted group were less than in the control group. Our findings suggest that transplantation of autogenous chondrocytes, cultured in atelocollagen gel, may improve treatment of the injured growth plate.

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To prevent arrest of growth, after physeal injury, several studies have transferred fat (Langenskiöld 1967, Österman 1972), muscle (Martiana et al. 1996), polymeric silicone (Cambell et al. 1959, Bright 1974, Macksoud and Bright 1989, Lee et al. 1993), bone wax (Broughton et al. 1989), and bone cement (Klassen 1982) as interposition materials after resecting the bone-bridge. Langenskiöld (1967) reported that the implantation of autogenous fat tissue after resecting a bone-bridge prevented the reformation of any bone-bridge for a long time; this permitted correction of the deformity by growth. Although this procedure has been successful in clinical work, it has proved useful only when the extent of the injury is relatively limited and the growth plate has a framework strong enough to provide structural support even after the bone-bridge is resected (Langenskiöld et al. 1986).

Foster et al. (1990) reported the use of cultured chondrocytes in collagen gel for repair of a physeal defect in a sheep model. This prevented the formation of a bone-bridge in a small physeal defect (less than 20% of the total physis). Using a rabbit model, Lee et al. (1998) modified their method to culture allogeneic chondrocytes embedded in agarose instead of collagen. They showed that chondrocytes in agarose could also reduce the leg-length discrepancy and angular deformity after a larger physeal arrest (50% of the physis). However, agarose has not been used clinically and its clinical safety is uncertain. Furthermore, allogeneic chondrocytes are problematic, not only in terms of their antigenicity, but also because of the risk of transmissable diseases.

We have previously reported a good outcome after treating articular cartilage defects with transplantation of autogenous cultured chondrocytes

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embedded in atelocollagen gel. This gel has low immunogenicity and is safe because immunogenic telopeptide is removed from the collagen (Ochi et al. 1998). In an animal model, we showed that grafted chondrocytes embedded in atelocollagen gel could synthesize the extracellular matrix in the defect for a long time after transplantation, surviving bone replacement after invasion by bone marrow cells (Katsube et al. 2000).

In the present study, we evaluated the transplantation of autogenous chondrocytes, cultured in atelocollagen gel, for treatment of a growth plate injury.

Animals and methods

Chondrocyte isolation and culture

Articular cartilage slices (5 × 5 mm) were removed with a surgical knife under sterile conditions from the unloaded region of the femoral condyle in the knee joints of 8–10-week-old Japanese white rabbits. Cartilage specimens were minced and washed three times in physiological saline. Chondrocytes were then isolated. by using 0.25% trypsin (Difco Lab, Michigan, USA) in sterile saline for 30 minutes and 0.25% collagenase (CLS II, New Jersey, USA) in Dulbecco's modified Eagle's medium (DMEM, Nissui Co Ltd, Tokyo, Japan). supplemented with 10% fetal calf serum (FCS, JR Scientific, CA, USA), HEPES buffer (10 nmol/L), 1% antibiotics (10,000 U/mL penicillin, 10 mg/mL streptomycin and 25 μg/mL Fungizone; Biowhittaker Inc, Maryland, USA) for 6 hours at 37 °C in a culture tube. The isolated chondrocytes were washed three times with culture medium and counted in a hemocytometer. The chondrocytes were embedded in atelocollagen (atelocollagen; 3% type I collagen, Koken Co Ltd, Tokyo, Japan) containing the culture medium. 400 mL of this medium-cell collagen mixture were placed in a 20-mm diameter culture dish and allowed to gel in a carbon dioxide incubator for 10 minutes at 37 °C. The gel was overlaid with 2.0 mL of the culture medium. Cell cultures were incubated in a mixture of 5% CO_2 and 95% air at 37 °C for a week. The medium was changed every 3 days and L-ascorbic acid (50 μg/mL)was added every other day. After being cultured for 1 week, this chondrocyte-gel complex was transferred back to the same rabbits.

Creation of growth arrest and transplantation of chondrocytes

We used 40 immature 8–10-week-old male Japanese white rabbits weighing 1.5–1.8 kg. The growth plate of these animals fuses at 6–7 months of age. Surgery was performed under combined general anesthesia, using 3 mL ketamine hydrochrolide and 3 mL xylazine given intramuscularly. In each rabbit, both knees were shaved and disinfected with 70% alcohol. After an anteromedial incision on the left tibia, the medial two thirds of the proximal physeal plate of the tibia was exposed and excised with a no. 15 scalpel blade and a small curette, resulting in a physeal defect. Our preliminary experiment showed that this procedure produced a bone-bridge in the physeal plate, causing growth arrest. The animals were then randomly divided into 3 groups. For the transplantation of chondrocytes (Ch group), the defect was filled with chondrocytes cultured in the atelocollagen gel for 1 week. For the transplantation of autogenous fat tissue (Fat group), the latter was taken from the groin and grafted into the defect. In the other control group (Defect group), the defect was left empty. We allocated 12 animals to each group preliminarily, and substitituted 4 animals for those that had been excluded because of infection (Ch group: 1, Fat group: 2, and Defect group: 1). After the operation, they were allowed to walk freely in the cages without a splint. Of 12 rabbits in each group, radiographs were taken in 6 animals up to 52 weeks after surgery, while tissues of the remaining 6 animals were examined histologically.

Histological evaluation

The animals were killed at 4, 12, and 52 weeks after surgery with sodium pentobarbital (70 mg/kg). Tissues were taken for histological examination from 3 animals in each group at 4 and 12 weeks after surgery, and from 6 animals in each group at 52 weeks after the radiological examinations. The tibia was excised and fixed in 10% buffered formalin for 10 days. Each specimen was decalcified in 2.5% formic acid buffered with citric acid and embedded in paraffin. Sections 7 μm thick were cut through the grafted area sagittally, and stained with safranin O, fast green and iron hematoxylin. Numerous histological sections of the grafted area were examined for the quality of the grafted tissue.

Radiographic assessments

The growth plate of the left tibia was oriented in an anteroposterior X-P direction for quantitative evaluation of angular deformity and length discrepancy before and 2, 4, 8, 12, 24 and 52 weeks after surgery. The angular deformity, subtracting 90° from the angle inclined between the long axis of the tibia and tibial plateau, was called angle α. As regards the length discrepancy, it was calculated by subtracting the length of the midline of the operated side of the left tibia from that of the unoperated side of the right tibia.

Statistics

The statistical analysis was done using the Statview 4.5 (Abacus, Barkely, CA, USA) program. Values are given as means (SD) of the group. The radiographic data (the angular deformity and length discrepancy) were analyzed with a two-way ANOVA as regards treatment and time. P-values less than 0.05 were considered statistically significant.

Results

Histological findings (Figure 1)

In the Ch group, the columnar arrangement of grafted chondrocytes and the extracellular matrix in the region surrounding them were visible at 4 weeks after surgery. At 12 weeks, ramified vascularization and ossification were found in the marginal area of the grafted chondrocyte-gel complex. However, both the grafted chondrocyte-gel complex and residual growth plate were almost intact, although the edge of the growth plate which came into contact with this gel inclined to the chondrocyte-gel complex side. We also found that physeal ossification had continued in the uninjured growth plate. At 52 weeks after surgery, most of the transplanted chondrocyte-gel complexes were replaced by bone and the residual growth plate had probably closed.

In the Fat group, at 4 weeks after surgery the defect was filled with living fatty tissue accompanied by connective tissue and blood vessels. At 12 weeks, the grafted fatty tissue had retained its histological appearance. Even at 52 weeks, transplanted fat tissue was alive and had expanded with bone growth.

In the Defect group, the cavity had collapsed and very little space was visible. At 4 weeks after surgery, the residual space had filled with connective tissue and blood vessels. At 12 weeks, because of bone-bridge formation between the epiphysis and metaphysis, the cavity had completely collapsed, but the residual physeal plate had survived. At 52 weeks, the growth plate showed complete ossification of the physis, which was slanted.

Radiographic assessments

Angular deformity. At 2 weeks after surgery, the angular deformity in the Ch and Fat groups was smaller than that in the Defect group. However, at 4 weeks, the Fat group had a greater increase in angular deformity than the Ch group. The angular deformity in the Ch group remained smaller than that in the other groups from 8 weeks until 52 weeks after surgery. The angular deformity α in the Ch, Fat, and Defect groups was then 15 (4)°, 25 (8)°, and 24 (4)°, respectively. The Ch group showed by far the smallest angular deformity of the three groups (Figures 2 and 3) ($p < 0.001$), and the change during the postoperative period also differed in each group ($p = 0.01$).

Length discrepancy. The length was less in the Ch group than in the other groups from 4 weeks until the last examination (52 weeks) after surgery, although it gradually increased during this period. At 4 weeks postoperatively, the length discrepancy in the Fat group was relatively less than that in the Defect group, but it increased rapidly and exceeded that in the Ch group from 12 weeks. Thus, at 52 weeks after surgery, the length discrepancies were 12 (3) mm in the Ch group versus 17 (2) mm and 16 (2) mm in the Defect and Fat groups, respectively (Figures 2 and 4). The Ch group showed by far the least was length discrepancy of the three groups ($p < 0.001$), and the change during the postoperative period also differed in each group ($p < 0.001$).

Discussion

We found that transplantation of autologous chondrocytes cultured in atelocollagen gel reduced the angular deformity and length discrepancy of the leg with the injured physis, more than transplanta-

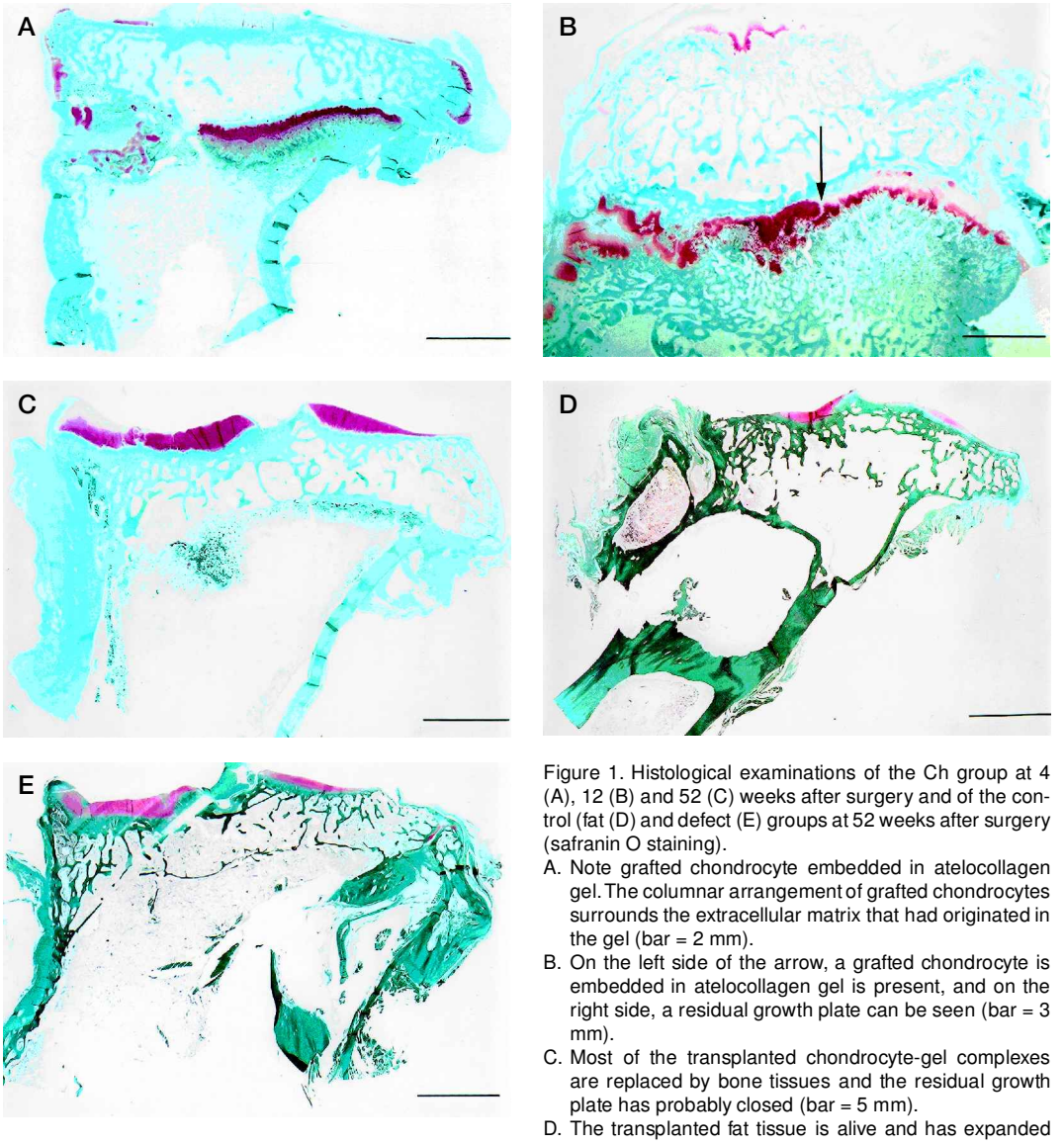

Figure 1. Histological examinations of the Ch group at 4 (A), 12 (B) and 52 (C) weeks after surgery and of the control (fat (D) and defect (E) groups at 52 weeks after surgery (safranin O staining).

A. Note grafted chondrocyte embedded in atelocollagen gel. The columnar arrangement of grafted chondrocytes surrounds the extracellular matrix that had originated in the gel (bar = 2 mm).
B. On the left side of the arrow, a grafted chondrocyte is embedded in atelocollagen gel is present, and on the right side, a residual growth plate can be seen (bar = 3 mm).
C. Most of the transplanted chondrocyte-gel complexes are replaced by bone tissues and the residual growth plate has probably closed (bar = 5 mm).
D. The transplanted fat tissue is alive and has expanded longitudinally with bone growth, but it has shortened horizontally (bar = 5 mm).
E. The growth plate shows complete ossification of the physis which is slanted (bar = 5 mm).

tion of fat. We suggest the following reasons for these good results.

Chondrocytes embedded in atelocollagen gel may give better mechanical support than fat to prevent a collapse with time. It is uncertain whether chondrocytes containing gel acquires sufficient mechanical stiffness at transplantation, but transplanted chondrocytes can proliferate and synthesize the extracellular matrix sometime after transplantation, thereby providing a stiffer interposition material. Finally, transplanted chondrocytes can be replaced with bone and united by ossification at the uninjured growth plate. Radiographs also showed that the postoperative angular deformity and length discrepancy may have been smaller. In contrast, in the case of fat tissue, the stiffness of the transplanted fat may be insufficiently developed after transplantation, and lead to collapse of the transplanted material. Therefore, the postoperative angular deformity and length discrepancy gradually became larger, and the transplanted fat

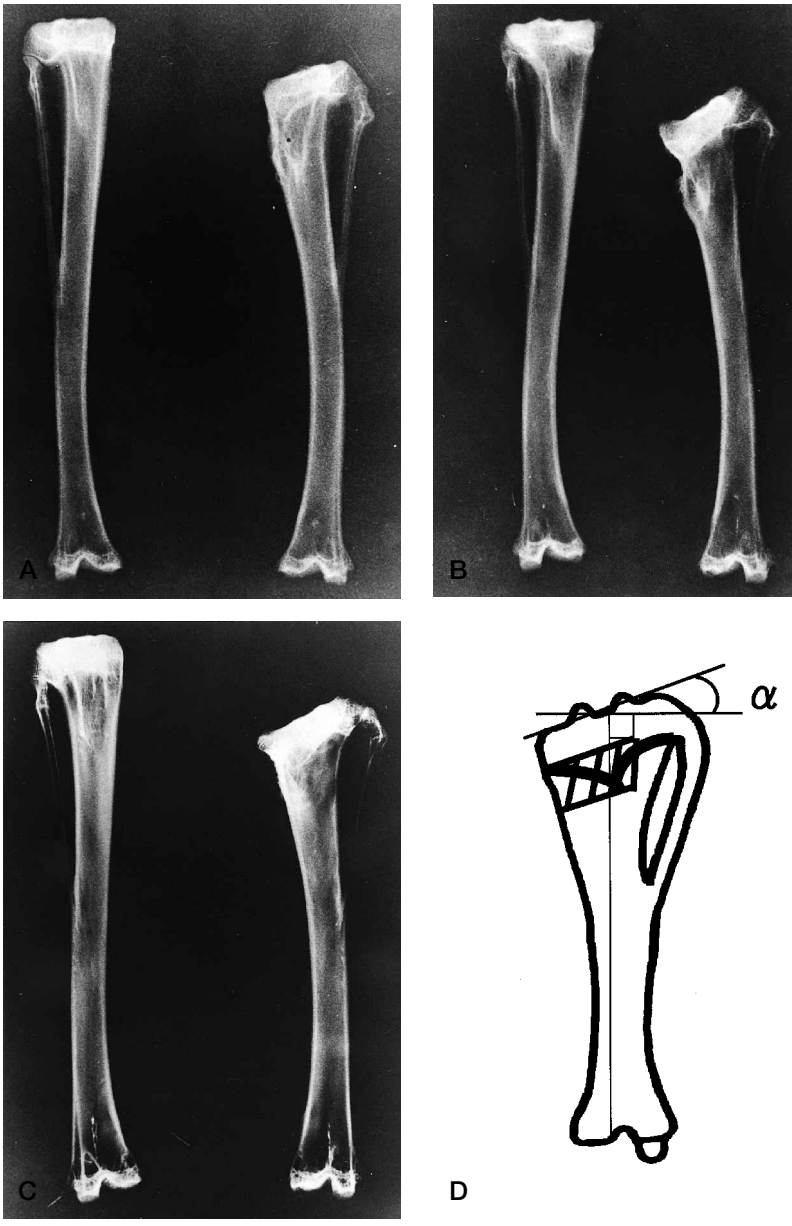

Figure 2. The radiographic findings at 52 weeks after surgery and the definition of angle α.
A. Ch group.
B. Fat group.
C. Defect group. Angular deformity is ranked: (A)<(C)<(B), length discrepancy is ranked: (A)<(B)<(C).
D. The angular deformity is shown as angle α. It is measured by subtracting 90° from the angle between the long axis of the tibia and the tibial plateau, which expresses the degree of inversion.

tissue remained even after closure had developed in the growth plates (Langenskiöld et al. 1986), confirming the presence of weak points at the transplanted site. Chondrocyte transplantation will prevent bridge formation, but not provide sufficient mechanical support to prevent substantial angular deformity. In a clinical setting, therefore, additional mechanical support will be needed.

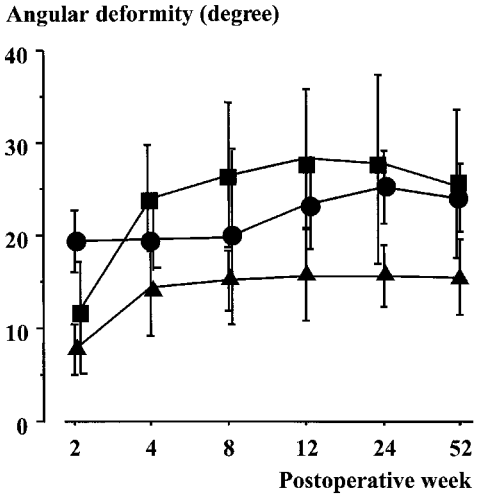

Figure 3. Consecutive changes in angular deformity in the Ch (▲), Fat (■), and Defect (●) groups.

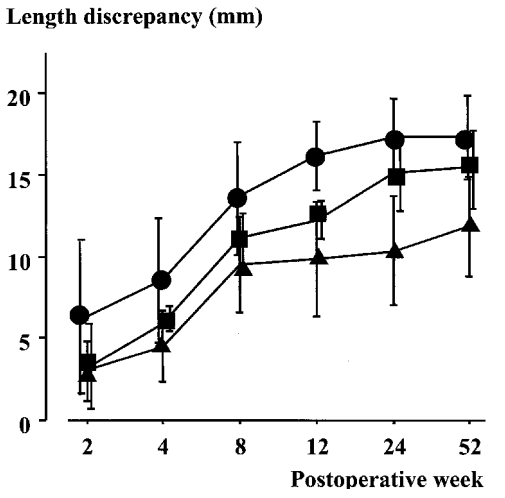

Figure 4. Consecutive changes in length discrepancy in the Ch (▲), Fat (■), and Defect (●) groups.

Since Langenskiöld's report (1967), fat has been used successfully in clinical work as an interposition material to prevent the formation of new bone bridges. Our study also showed that fat can prevent the formation of bridges across the epiphysis and metaphysis even when two thirds of the growth plate have been resected. Therefore, the use of fat may be effective for treating a physis with relatively little damage, that needs less mechanical support at the resection site. In the cases with large bone-bridges, the interposition material should give mechanical support to prevent collapse at the resection site. Our findings suggest that autologous chondrocyte transplantation may therefore be effective in cases where the growth plate must be extensively resected.

According to Martiana et al. (1996), the results with non-biological grafts for extensive growth plate injuries have been worse than those with biological grafts. This is because the graft materials also must have high biological tissue-like affinity to the surrounding tissues of the transplant site. Apart from cartilage, fat and muscle have been evaluated as biological materials. As regards fat and muscle tissues, insufficient stiffness is commonly thought to disqualify them, since it weakens the grafted material. In transplant cases using cartilage, autogenous iliac apophysis (Olin et al. 1984) and allogeneic chondrocytes (Foster et al. 1990, Lee et al. 1998) have been tried. A bone-bridge forms in sites where a dead space has developed between the uninjured growth plate and graft shortly after transplantation. Furthermore, although excellent progress has been made with the allogeneic chondrocyte graft at 4 (Foster et al. 1990) and 16 (Lee et al. 1998) weeks after surgery, the final outcomes may be poor, due to immunoresponse-related problems generated over a long period (Stevenson et al. 1989). Recently, highly transmissible diseases, such as AIDS, have complicated the use of these grafts (Asselmeier et al. 1993).

Even when autologous chondrocyte transplantation was used, angular deformity and leg discrepancy occurred. This suggests that even when this technique is used, one can not rule out the need for corrective osteotomies and/or leg-lengthening procedures after growth is complete. A long period without weight-bearing after transplantation may enable grafted chondrocytes to synthesize a greater amount of extracellar matrix and the grafted material to become stiffer, leading to better results than those in these animal experiments. In conclusion, the use of autogenous chondrocyte transplantation may be better than autogenous fat tissue for cases in which the damaged area is larger than two thirds of the physis.

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