Transplantation of osteoblast-like cells to the distracted callus in rabbits

S. Tsubota, H. Tsuchiya, Y. Shinokawa, K. Tomita, H. Minato

From Kanazawa University, Japan

We carried out limb lengthening in rabbits and then transplanted osteoblast-like cells derived from the tibial periosteum to the centres of distracted callus immediately after distraction had been terminated. Two weeks later the transaxial area ratio at the centre of the distracted callus and the bone mineral density (BMD) were significantly higher in the transplanted group, by 21% and 42%, respectively, than in the non-injected group or the group injected with physiological saline (p < 0.05). Callus BMD as a percentage of density in uninvolved bone was also significantly higher in the transplanted group (p < 0.05) than in the other two groups, by 27% and 20% in the second and fourth weeks, respectively (p < 0.05). Mechanically, the callus in the transplanted group tended to be stronger as shown by the three-point bending test although the difference in fracture strength was not statistically significant.

Our results show that transplantation of osteoblast-like cells promotes maturity of the distracted callus as observed at the second and fourth weeks after lengthening. The method appears promising as a means of shortening the consolidation period of callus distraction and decreasing complications during limb lengthening with an external fixator.

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The importance of distraction osteogenesis is now well recognised. However, to achieve extensive bone regeneration the external fixator must be applied for a consider-

able time, which may result in many complications. Shortening of the period of external fixation would reduce costs and the incidence of complications. Our aim was to demonstrate the effect of transplantation of osteoblast-like cells on the maturation of the distracted callus in rabbits.

Materials and Methods

Experimental design. We performed limb lengthening on 54 young male Japanese white rabbits weighing between 2.5 and 3.2 kg. They were anaesthetised with an intramuscular injection of ketamine hydrochloride (50 mg/kg body-weight; Warner-Lambert, Morris Plains, New Jersey) and an intravenous injection of pentobarbital sodium (40 to 50 mg/kg body-weight; Abbott Laboratories, North Chicago, Illinois). Each animal was placed supine on the operating table; the right hind leg was sterilised with povidone iodine and draped to expose the calf. An 8 cm skin incision was made on the anteromedial aspect of the tibia, which was then exposed subperiosteally. Four half pins of 2 mm diameter (Howmedica, Geneva, Switzerland) were inserted perpendicular to the axis of the tibia and a hemilateral dynamic external fixator of our design was applied. The tibia was osteotomised at the distal margin of the tibiofibular junction with a thread wire saw of 0.36 mm diameter (Koshiya, Kanazawa, Japan), and gradually lengthened at a rate of 1 mm per day by adjusting the lengthener once daily for 20 days, starting seven days after the operation.

We then divided the rabbits into three groups: group I (22 rabbits) had callus distraction only with no injection, group II (12 rabbits) had injection of physiological saline (0.5 ml) into the centre of the callus immediately after distraction (12 rabbits) and group III (20 rabbits) had transplantation of osteoblast-like cells derived from the tibial periosteum (5 x 10^6 cells in 0.5 ml of physiological saline) (Table I).

<table>
<thead>
<tr>
<th>Table I. Details of the experimental design</th>
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<tr>
<td>Group</td>
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<tr>
<td>I (non-injected)</td>
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<td>II (saline)</td>
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<td>III (osteoblast-like cells)</td>
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Correspondence should be sent to Dr H. Tsuchiya.

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The animals were then killed every two weeks for eight weeks beginning after the termination of lengthening. The harvested specimens were evaluated by histology, morphometry, radiography, dual-energy X-ray absorptiometry (DEXA) and a three-point bending test (Table II).

**Cell cultures.** We used the technique of Jones and Boyde for the culture of osteoblast-like cells. The periosteum was stripped from the proximal surface of both tibiae over an area of 8 × 12 mm during the operation. It was washed three times with agitation in Earle’s salts for ten minutes, then in a solution of 500 U/ml of penicillin and 0.5 mg/ml of streptomycin for five minutes, and finally once more in Earle’s salts. The strips were then cut into rectangles of 2 to 3 mm. Small pieces (area 2 cm²) were placed with the osteogenic layer downwards directly on to the surface of a 60 mm Petri dish and covered with warm Dulbecco’s modified Eagle’s medium (Gibco Life Technologies, Grand Island, New York) to which 10% heat-inactivated fetal bovine serum (JRH Biosciences, Lenexa, Kansas) and 200 mg/l of ascorbic acid had been added. They were incubated at 37°C in an atmosphere containing 21% O₂ and 5% CO₂ with the tissue-culture medium being sustained by each bone. The specimens were placed in physiological saline of 5 cm depth during measurement. The results were expressed as the bone mineral density (BMD, mg/cm³), specifically as the ratio of bone mineral content to the coronal area of the distracted callus. The percentage of the BMD is the ratio of the mean BMD in the lengthened portion (C) to that in the distal (D) and proximal (P) parts of the unlengthened tibial shaft at 5 mm from the line of osteotomy, and is expressed by the formula \( \frac{C}{P + C/D} \times \frac{1}{2} \times 100 \)."
Radiological analysis. The mean actual length achieved was 20 ± 1.0 mm in all groups. Radiography showed that the distracted callus of group III was thicker and more solid than that of the other two groups (Fig. 2).

Bone mineral analysis. The time course of changes in the BMD for each of the experimental groups measured by DEXA after termination of lengthening is shown in Figure 3. While the BMD of groups I and II reached a maximum in the fourth week, that of group III did so in the second week. In all groups the BMD decreased in the sixth and eighth weeks. In the second week the BMD of group III was significantly higher (p < 0.05) than that of the other two groups. There were no significant differences in the BMD between groups I and II at any time. The time course of the changes in %BMD for each of the experimental groups is shown in Figure 4, indicating that there were no remarkable changes in %BMD in groups I and II after two weeks, but that it was significantly higher in group III (p < 0.05) than in the other two groups in the second and fourth weeks. No significant differences were found in the

![Graph](image1.png)

Fig. 1

The transaxial area ratio (%) in the three groups over the experimental period (*p < 0.05).

![Radiographs](image2.png)

Fig. 2

Radiographs of tibiae from a) group I (callus distraction only, no injection) and b) group III (transplantation of osteoblast-like cells derived from the tibial periosteum) showing distracted callus in the second week after termination of lengthening.

![Graphs](image3.png)

Fig. 3

Figure 3 – Time course of the changes in BMD measured by DEXA after termination of lengthening. The BMD of group III (transplanted group) was significantly higher than that of the other two groups (non-injected group and saline group) in the second week (*, p < 0.05). Figure 4 – Time course of the changes in %BMD. There was a significant difference between group III and the other two groups in the second and fourth weeks (*: p < 0.05).

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%BMD between the other two groups at any time. In group III it reached a maximum in the fourth week and decreased to the level of the other two groups after the sixth week.

**Mechanical analysis.** The time course of changes in fracture strength as measured by the three-point bending test in each experimental group is shown in Figure 5. In all groups the value reached a maximum in the fourth week and decreased after six to eight weeks. No significant differences were seen among the three groups at any time.

**Histological analysis.** The histological findings of group I were similar to those described previously.13,14 Immediately after lengthening had been terminated, the central radiolucent area between the proximal and distal calcified trabecular area was composed mainly of masses of fibroblast-like cells with abundant small vessels. The proximal and distal ends of the longitudinal fibres bridging the radiolucent area merged with fibrocartilage which contained hypertrophic chondrocytes arranged in a columnar fashion. New bone was formed in the adjacent sclerotic zone. Group III showed evidence of more longitudinal bony trabeculae as stained by haematoxylin and eosin (Fig. 6), more proteoglycan as stained by Toluidine Blue, and more collagen fibres as stained by the Azan-Mallory method than did group I in the second week. There were no islands of transplanted osteoblast-like cells or formation of new bone in the central fibrous tissue.

**Discussion**

Complications during and after limb lengthening include delayed consolidation, nonunion, late bowing and refracture. The incidence and severity of these complications tend to increase in relation to the period of external fixation, proportionately reducing the likelihood of achieving the functional objective of surgery.15,16 Attempts have been made to promote maturity of the distracted callus, such as the use of periodic and static compression and electrical stimulation, the administration of agents such as vitamin D, macrophage-colony stimulating factor or bisphosphonate, and transplantation of bone-marrow cells.11 It is now possible to culture osteoblast-like cells derived from cortical bone, cancellous bone and periosteum,9,10,17 and some attempts at promoting fracture healing and allograft enhancement have been undertaken using such cells.18 We have used this method in rabbits to hasten the maturity of the distracted callus.
of distracted callus and to shorten the period of external fixation with its exposure to complications.

Our study has shown that in the group with osteoblast-like cell transplantation, the transaxial area ratio was 21% higher (p < 0.05, Fig. 1) and the BMD 42% higher in the second week (p < 0.05, Fig. 3) than in the control groups. The %BMD was also 27% higher than that of the control groups in the second week and 20% more in the fourth week (p < 0.05, Fig. 4).

The volume of new bone and the amount of bone mineral in the distracted callus were increased by osteoblast-like cell transplantation in the early stage of limb lengthening. We speculate that this procedure has two different effects: it increases the number of osteogenic cells in the distracted callus and stimulates networks for membranous and cartilaginous osteogenesis via cytokines, collagen and other agents derived from the transplanted cells. The specific mechanisms by which osteoblast-like cell transplantation produces these effects remain to be elucidated, and more detailed experiments are needed to identify the optimum conditions of transplantation. Our results have shown, however, that in the rabbit osteoblast-like cell transplantation promotes the maturity of distracted callus in the early stage after lengthening, which, if successful in man, could shorten the consolidation period of callus distraction and the overall period of external fixation.

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No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References