cases, cells are dissociated from pieces of articular cartilage, propagated (or left unpropagated) on dishes in ex vivo conditions to expand the cell population, and then transplanted with or without scaffolding carrier materials into the cartilage defect of the recipient. Although these methods can repair cartilage defects, some difficulties persist. Allogeneic transplantation has the inherent risks of disease transmission and rejection; autologous transplantation causes damage to the donor site.

In an effort to address the limitations of existing approaches, we attempted to generate cartilage tissue by inducing the differentiation of muscle-derived cells into the chondrocytic lineage in an in vivo environment with recombinant human bone morphogenetic protein 2 (rHuBMP-2). Articular defects in rat joints that received the induced cartilage-like tissue were repaired and restored to normal condition. The present report provides evidence to support this approach for the successful treatment of articular cartilage defects.

MATERIALS AND METHODS

Preparation of muscle-derived mesenchymal cells and diffusion chambers. Mesenchymal cells were obtained from the thigh muscles of 19-day, postcoital, F344 rat embryos (purchased from Japan SLC, Hamamatsu, Japan). The muscle tissues were minced with scissors and digested in 0.25% trypsin with 1 m*M* EDTA-Na_4 (Invitrogen, Carlsbad, CA). The dissociated cells were propagated on plastic culture dishes (10 cm in diameter) in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% (volume/volume) fetal calf serum (Invitrogen) and antibiotics (mixture of 5 mg/ml penicillin G, 5 mg/ml streptomycin, 10 mg/ml neomycin; Invitrogen) and passaged under routine culture conditions for 10 days. At the end of this period, the cells were detached from the dishes with 0.25% trypsin with 1 m*M* EDTA-Na_4 and packed within diffusion chambers (10^6 cells/chamber).

In order to construct a diffusion chamber for cell transplantation, a diffusion chamber kit (Millipore, Billerica, MA), consisting of a plastic ring (14 mm in outer diameter and 10 mm in inner diameter), a membrane filter (comprising a mixture of cellulose acetate and cellulose nitrate [0.45 μm in pore size]), and adhesive sealant, was utilized. The inner diameter of the ring was reduced to 5 mm by inserting another plastic ring. Only one side of the larger plastic ring was initially sealed with a membrane filter and adhesive sealant. For the next step, 40 μl of 0.3% (weight/weight) pig type I collagen (Cellmatrix LA; Nitta Gelatin, Osaka, Japan) and 0, 1, or 10 μg of rHuBMP-2 (Yamanouchi Pharmaceutical, Tokyo, Japan) were introduced into the diffusion chamber. The chamber was then freeze-dried and sterilized with ethylene oxide gas.

After these processes were completed, 10^6 cells suspended in 40 μl of serum-free culture medium containing 0.3% (w/w) pig type I collagen (Cellmatrix I-A; Nitta Gelatin) were introduced into the diffusion chamber, and another open side of the chamber was sealed with a filter and adhesive sealant. Sixty-two chambers (42 for histologic examination, 8 for reverse transcription–polymerase chain reaction [RT-PCR] analysis, and 12 for real-time PCR analysis) with 10 μg of rHuBMP-2 (group B10), 10 chambers (all for histologic examination) with 1 μg of rHuBMP-2 (group B1), and 46 chambers (26 for histologic examination, 8 for RT-PCR analysis, and 12 for real-time PCR analysis) without rHuBMP-2 (group B0) were prepared for analysis and implantation.

Transplantation of the diffusion chamber into the abdominal pocket of rats. Immediately after loading the cells into the diffusion chambers, each chamber was surgically inserted into a pocket in the abdominal muscles of 8-week-old F344 rats under diethyl ether anesthesia. After surgery, the rats were housed in cages and were given free access to standard chalk-like food and water. At 2, 4, 6, 8, 14, 21, 28, 35, and 42 days after implantation, the animals were killed in due order and the diffusion chambers were harvested (Table 1) for histologic examination. For RT-PCR analysis, 2 chambers were harvested at 2-, 4-, 7-, and 14-day intervals after implantation. For real-time PCR analysis, 2 chambers were harvested at 2-, 4-, 6-, 14-, 28-, and 42-day intervals after implantation.

Harvested tissue pellets within the chambers were inspected for vascular invasion caused by seal failure or breakage of the filter membranes. When vascular invasion was noted, the tissue was excluded from the transplantation into the cartilage defect and from PCR analysis. The tissue pellets for histologic examination were radiographed and fixed in 20% neutral buffered formalin solution, prior to processing for histologic examination. Some parts of the tissue pellet from the 5-week-old sample were used for transplantation into the rat-knee defect. Tissue pellets for RT-PCR or real-time PCR were frozen in liquid nitrogen immediately after harvesting.

Transplantation of tissue pellets from diffusion chambers into osteochondral defects of rats. Some portions of the tissue pellet removed from the diffusion chambers at 5 weeks after implantation were transplanted into cartilage defects generated on the patellar grooves of the knee joints of 7 (4 from group B10, 3 from group B0) mature, same-strain rats (a quarter tissue pellet/animal). The transplantation procedure was performed with the rats under anesthesia, using an intramuscular injection of a mixture of ketamin (100 mg/ml, 0.6

Table 1. Cartilage formation in diffusion chamber*

	rHuBMP-2			Area of cartilage tissue in cross-section
	0 μg	1 μg	10 μg	
2 days	0/2	-	0/2	-
4 days	0/2	-	0/2	-
6 days	0/2	-	0/2	-
8 days	0/2	-	0/2	-
14 days	0/2	-	0/2	-
21 days	0/4	-	4/6	1/4
28 days	0/4	0/4	9/10	1/3
35 days	0/4	0/6	9/10	Almost all
42 days	0/4	-	6/6	Almost all

* Except where indicated otherwise, values are the number of samples with cartilage formation/number of experiments. rHuBMP-2 = recombinant human bone morphogenetic protein 2.

ml/kg body weight; Sankyo, Tokyo, Japan) and xylazine (20 mg/ml, 0.3 ml/kg body weight; Bayel, Osaka, Japan). Pellets were transplanted into the left knees, and defects made on the right knees did not receive the implants.

In order to generate an osteochondral defect on the patellar groove of the distal femur of the rats, a longitudinal skin incision was made in the midline of the knee and the patellar groove was exposed by medial parapatellar arthrotomy and lateral dislocation of the patella. The osteochondral defect was made by drilling in 2 mm in depth and 2 mm in diameter, vertically to the patellar groove. The tissue pellet was detached from the inner surface of the membrane filters of the diffusion chamber and press-fitted into the defect. The knee joint was then closed with sutures. After surgery, the rats were fed in cages and killed at 24 weeks after surgery. The knee joints were excised and processed for histologic examination.

Histologic examination. Diffusion chambers and distal femurs with an articular cartilage defect were removed from the animals at 24 weeks after implantation and fixed in 20% buffered formalin. The harvested chambers were radiographed with a soft x-ray apparatus (Sofron, Tokyo, Japan) and visualized on radiographic films (Fuji Photo Film, Tokyo, Japan). The harvested chambers with calcified tissue and the distal ends of femurs with articular defects were decalcified in 4% EDTA solution, and then dehydrated with a gradient ethanol series, embedded in paraffin, sectioned in 5-μm thickness, and stained with hematoxylin and eosin or toluidine blue. Results of the histologic examination were evaluated using the scoring system described by Wakitani et al (13) for histologic grading of a cartilage defect (Wakitani's score; a lower score indicates improvement).

RT-PCR analysis. In order to detect changes in the expression of cartilage matrix–specific molecules in cells from the harvested diffusion chambers, RT-PCR analyses for aggrecan, types II, IX, X, and XI collagens, MyoD1, and core binding factor a1 (Cbfa1)/runt-related gene 2 (Runx2) were performed with the tissue pellets from the B10 and B0 groups. Frozen tissue pellets were ground down to powder with liquid nitrogen in a mortar on dry ice, and total messenger RNA (mRNA) was extracted from the tissue using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. After treating samples with RNase-free deoxyribonuclease I (Takara Bio, Otsu, Japan), 500 ng of total mRNA from each sample was reverse transcribed using SuperScript II (Invitrogen). The reaction time was 60 minutes at 42°C. Thereafter, 1 μl of each reaction product was amplified in a 15-μl PCR mixture containing 0.5 units TaKaRa EX Taq (Takara Bio) and 10 pmoles of each primer to detect mRNA specific to each molecule.

Amplifications were performed in a Program Temp Control System (DNA Engine PTC-200; MJ Research, Waltham, MA) for 35 cycles after an initial denaturation step at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing for 30 seconds at 60°C, and extension at 72°C for 30 seconds, with a final extension at 72°C for 3 minutes. The PCR products (10 μl) were electrophoresed in a 3% agarose gel and detected by ethidium bromide staining. The nucleotide sequences of the primers for each of these genes are as follows: for AGC1, 5′-TCCAAACCAACCCGACAAT-3′ (forward) and 5′-TTCTGCCCAAGGGTTCTG-3′ (reverse); for Col2A1, 5′-GCTCGAGGAGACACTGGTG-3′ (forward) and 5′-ACCTGGGGGACCATCAGA-3′ (reverse); for Col9A1, 5′-GGTCCTCCGGGGAAGCCT-3′ (forward) and 5′-CCAACCTCTCCCGGCGGT-3′ (reverse); for Col10A1, 5′-CGAGGTCTTGTTGGCCCTAC-3′ (forward) and 5′-CCTGGGTCTCTGTCCGCT-3′ (reverse); for Col11A1, 5′-ATTGCCACCAGTCAACTGCT-3′ (forward) and 5′-TTGGACTGTGCCTCCGTC-3′ (reverse); for MyoD1, 5′-ACTACAGCGGCGACTCAGAC-3′ (forward) and 5′-GTGGAGATGCGCTCCACTAT-3′ (reverse); and for Cbfa1/Runx2, 5′-TGCTTCATTCGCCTCACAAAC-3′ (forward) and 5′-TAGAACTTGTGCCCTCTGTTG-3′ (reverse).

Real-time quantitative RT-PCR. Quantitative RT-PCR assay for type II collagen was carried out with the use of gene-specific expression–labeled fluorescent probes and sets of specific primers in an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA). On the basis of the published sequence of rat type II collagen, specific primer pair and probe sets were designed with the aid of Primer Express software, version 2.0 (Applied Biosystems). The sequences of the primers were 5′-AGGCGCTTCTGGTAACCCA-3′ (forward) and 5′-GACCAGTTGCACCTTGAGGAC-3′ (reverse), and the probe was 5′-TTCCCGGAGCCAAAGGATCTGCTG-3′. We used 6-carboxyfluorescein for type II collagen as the 5′ fluorescent reporter for the probe, while we added 6-carboxy-tetramethylrhodamine (Tamura Pharmaceutical, Osaka, Japan) to the 3′ end as a quencher.

Standard curves were constructed with the use of dilutions of accurately determined pCR2.1 plasmid vector (Invitrogen) containing complementary DNA (cDNA) products of type II collagen. A relative standard curve representing 10-fold dilutions of a rat type II collagen cDNA ranging from 2×10 to 2×10^5 copies/μl was used for linear regression analysis of the samples. PCR was carried out in 50 μl of reaction mixture containing 3 μl of the RT reaction, 1× Universal Master Mixture (Applied Biosystems), 500 n*M* of each primer, and 200 n*M* of the Taqman probe purchased from Applied Biosystems.

To compensate for the differences in cell number and/or RNA recovery, the copy number of type II collagen mRNA was determined relative to 18S ribosomal RNA (rRNA) (Applied Biosystems), which was also analyzed quantitatively. Thus, a partial cDNA of 18S rRNA was amplified from rat bone and cartilage samples using a specific primer set for 18S rRNA, and then subcloned into pCR2.1 (Invitrogen). Ten-fold dilutions of the resultant vector, pCR2.1-18S rRNA, ranging from 2×10 to 2×10^5 copies/μl, were used to construct a relative standard curve for 18S rRNA. The PCR mixture was basically the same as that for type II collagen, except for 200 n*M* of an 18S rRNA–specific Taqman probe set carrying a 5′-VIC reporter label and 3′-TAMURA quencher group, and 500 n*M* of the specific primer for 18S rRNA that was purchased from Applied Biosystems. These samples were placed in the ABI PRISM 7700 Sequence Analyzer and preheated at 95°C for 10 minutes, then amplified for 50 cycles of 95°C for 15 seconds, followed by 60°C for 1 minute. These experimental protocols were in compliance with the guidelines established by the Institutional Committee for Animal Care and Experiments of Shinshu University.

Statistical analysis. The histologic score was statistically analyzed using the SPSS software package (SPSS Japan, Tokyo, Japan). The Kruskal-Wallis H test followed by the

Mann-Whitney U test was used to determine differences between the groups.

RESULTS

Cartilage induction in diffusion chambers by rHuBMP-2. The tissue mass harvested from group B10 chambers (those receiving 10 μg rHuBMP-2) had a gelatinous appearance, with no histologic features characteristic of cartilage until 2 weeks after implantation. At 3 and 4 weeks after implantation, the tissue had a pale, opaque gelatinous appearance and revealed some cartilaginous characteristics along the inner surface of the filter membranes of the chamber on histologic examination (Figures 1A–H).

At 5 and 6 weeks postimplantation (Figures 1I–P), the cells of group B10 formed an elastic tissue mass with opaque appearance and no evidence of calcification on radiography (Figure 2B). Histologic examination of the opaque tissue mass in the chambers indicated normal features of cartilage, with round chondrocytic cells enclosed in a metachromatic matrix, as revealed by toluidine blue staining (Figures 1L and P). Small amounts of osseous tissue were found on the outer or host-side surfaces of the membrane filter of those samples. In one chamber with an accidental "hole" on the membrane filter, containing 5-week postimplantation tissue of group B10, the tissue became a hard mass with a reddish appearance; on radiography, the tissue was highly calcified (Figure 2C) and showed a normal histologic appearance of bone with hematopoietic marrow (Figure 2A). In contrast, the tissue of groups B0 (Figure 1) and B1 (chambers without rHuBMP-2 or with 1 μg rHuBMP-2, respectively) showed a gelatinous appearance with no histologic evidence of cartilage formation throughout the experimental period.

PCR findings. PCR analysis of the tissue in the diffusion chambers revealed a consistent expression of types X and XI collagen (Figure 3). Expression of type X collagen gradually increased in group B10. The expression of type II collagen was detected at low levels 2 days after implantation in group B10 (Figure 3). After 4 days, the expression of type II collagen was clearly detected in group B10. The expression of Cbfa1/Runx2 was clearly detected after 96

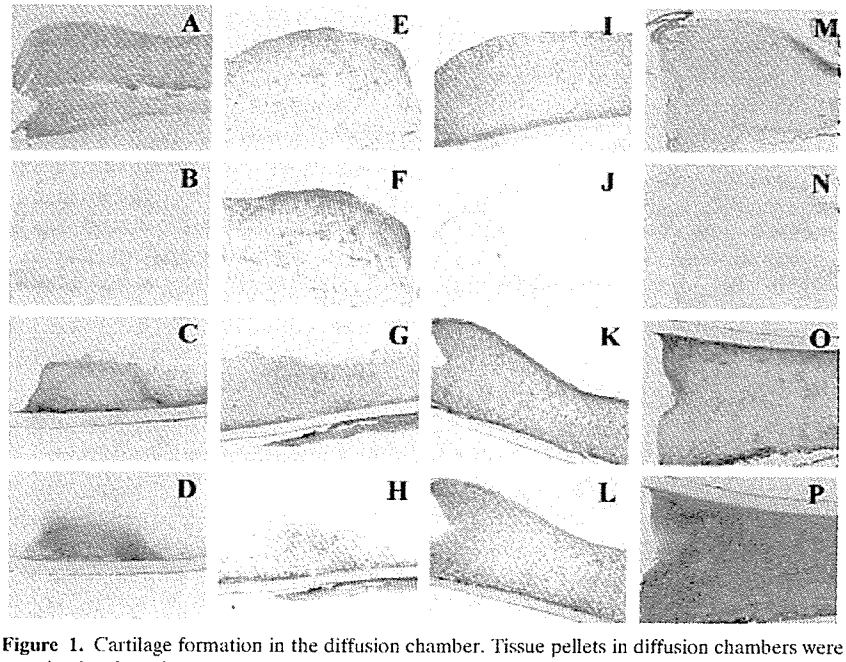

Figure 1. Cartilage formation in the diffusion chamber. Tissue pellets in diffusion chambers were examined at 3 weeks (**A–D**), 4 weeks (**E–H**), 5 weeks (**I–L**), and 6 weeks (**M–P**) postimplantation, in group B0 (without recombinant human bone morphogenetic protein 2 [rHuBMP-2]) (**A, B, E, F, I, J, M,** and **N**) compared with group B10 (with 10 μg rHuBMP-2) (**C, D, G, H, K, L, O,** and **P**). (Stained with hematoxylin and eosin in **A, C, E, G, I, K, M,** and **O**, with toluidine blue in **B, D, F, H, J, L, N,** and **P**; original magnification × 40.)

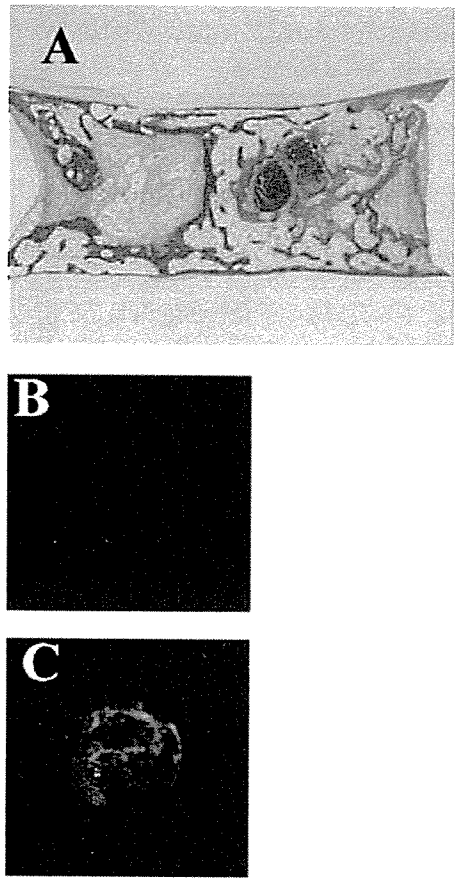

Figure 2. Histologic and radiologic evaluations of engineered cartilage tissue. For the tissue pellet in the diffusion chamber with an accidental hole on the filter (at 5 weeks posttransplantation; obtained from group B10), the normal histologic appearance of bone is clearly visible (stained with hematoxylin and eosin; original magnification ×20) (**A**), and the soft radiographic view shows bone trabeculae (**C**). Another soft radiographic view of group B10 tissue (same sample as in Figures 1K and L) shows no calcification (**B**).

hours in group B10 only (Figure 3). The expression of MyoD1 was not detected in either group at any time point.

Real-time PCR revealed that the expression of type II collagen increased markedly at 4 days after implantation (Figure 4). A high level of aggrecan was seen in group B10 after 2 days. Type IX collagen was weakly expressed in group B10 after 4 days, but increased significantly after 1 week. Low expression levels of aggrecan and type II collagen were detected in all groups at later time points in the study.

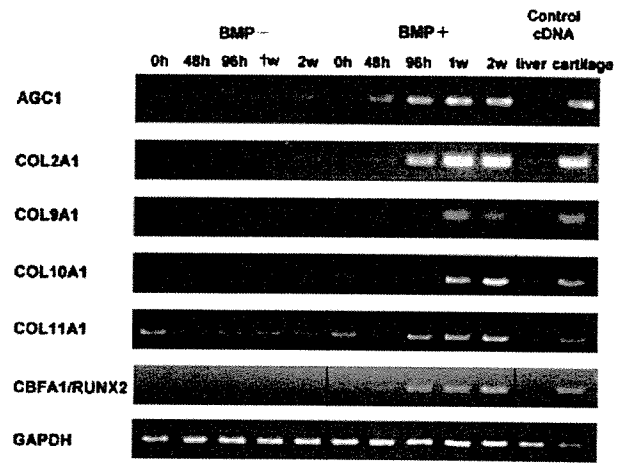

Figure 3. Reverse transcription–polymerase chain reaction analysis. Expression of types X and XI collagen (COL10A1 and COL11A1, respectively) was detected consistently in both groups (with 10 μg recombinant human bone morphogenetic protein 2 [BMP+; group B10] and without [BMP−]) throughout the experimental period. Expression of type IX collagen (COL9A1) was detected after 96 hours, indicating that effective cartilage matrix synthesis begins 3 or 4 days after implantation. Expression of type II collagen (COL2A1) was detected at low levels after 2 days in group B10 only, and after 4 days, it became more prominent. The expression of core binding factor a1/runt-related gene 2 (CBFA1/RUNX2) was clearly detected after 96 hours in group B10 only. AGC1 = aggrecan.

Repair of cartilage defects by transplantation of the engineered cartilage. The osteochondral defects that received the cartilaginous tissue mass, which was generated for 5 weeks in diffusion chambers containing tissue from group B10, were restored to a normal appearance at 24 weeks after transplantation. Upon examination, the site of the defects had a smooth surface and no

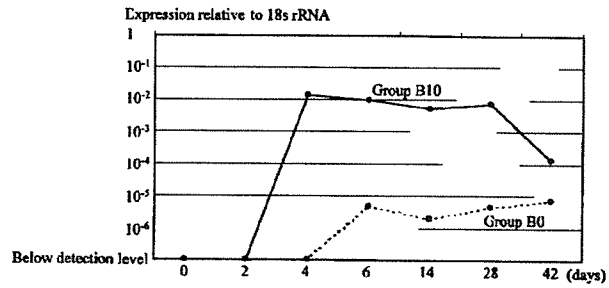

Figure 4. Real-time polymerase chain reaction analysis for type II collagen mRNA. After 4 days, expression of type II collagen mRNA was markedly increased in group B10 (with 10 μg recombinant human bone morphogenetic protein 2 [rHuBMP-2]). Group B0 = without rHuBMP-2; rRNA = ribosomal RNA.

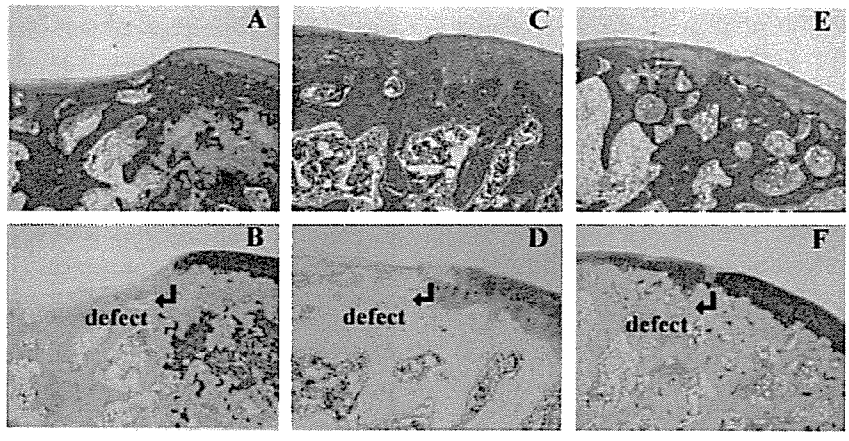

Figure 5. Osteochondral defects of a rat knee repaired with tissue pellets generated in diffusion chambers 24 weeks after transplantation. **A** and **B,** Defect with no implant. **C** and **D,** Defect implanted with tissue pellet generated in the chamber of group B0 (without recombinant human bone morphogenetic protein 2 [rHuBMP-2]). **E** and **F,** Defect implanted with tissue pellet generated in the chamber of group B10 (10 μg rHuBMP-2). (Stained with hematoxylin and eosin in **A, C,** and **E,** with toluidine blue in **B, D,** and **F;** original magnification × 40.)

obvious border with the surrounding normal articular cartilage (Figures 5E and F). The defects were filled with a layer of cartilage exhibiting subchondral cancellous bone connecting to the original subchondral bone. Although the architecture of the repaired articular cartilage was similar to that of normal cartilage with regard to cell arrangement, differences were noted. A tidemark was visible at the base of the cartilage layer adjacent to the subchondral bone, and the thickness of the regenerated cartilage was slightly less than that of the neighboring normal articular cartilage.

In contrast, the defects transplanted with tissue mass from group B0 were partially repaired, with a depressed surface visible at the defect site (Figures 5C and D). Histologic assessment of the defects that received either the tissue from group B0 or no implant revealed a small amount of fibrocartilage, with slightly positive metachromatic staining at the periphery of the defects and dominant fibrous tissue in the defect space.

Upon histologic evaluation of the knee cartilage after repair, the average histologic score (Wakitani's score) was 4.25 for group B10, 11.67 for group B0, and 14.00 for the defect-only group. The score for group B10 was significantly better than that for group B0 (P = 0.032) and the defect-only group (P = 0.002).

DISCUSSION

The experimental data presented herein indicate the capacity of rHuBMP-2 to induce the differentiation of young muscle-derived mesenchymal cells into chondrocytes within diffusion chambers in in vivo conditions. The resultant heterotopic cartilage formation represents a significant volume of induced tissue mass derived from these cells.

In order to induce the cartilage tissue, the diffusion chamber system was essential. When vascular invasion into the chamber occurred as a result of membrane seal failure, new bone with hematopoietic marrow was seen in the chambers harvested at 5 weeks after transplantation. Budenz and Bernard have reported similar findings (14). This bone was likely formed through the process of endochondral ossification, as deduced from classic reports describing the actions of BMP (15) and from comparison with the process of direct ossification (16,17). In the process of BMP-induced endochondral bone formation, cartilage is formed in the early phase of the bone-forming process. The cartilage tissue is then absorbed by invading vascular connective tissue and replaced by newly formed bone, as seen in embryonic osteogenesis (18) and in callus in fracture repair (19). During the process of ectopic bone formation elicited by

BMP, Tsuyama et al (20) found that the induced bone marrow cells are not the progeny of undifferentiated mesenchymal cells in situ, but rather arise from hematopoietic stem cells circulating in the peripheral blood. This conclusion was based on studies of chimeric mice and bone marrow transplantation (20).

In this diffusion chamber system, the cells within the chambers were able to survive by diffusion of tissue fluid from host animals, but vascular invasion was blocked by the filter membranes. As a result, the BMP-induced bone-forming reaction was stopped at the stage of cartilage formation and pieces of cartilage for transplantation were obtained, although it took a period of 5–6 weeks to achieve this outcome. This is a longer timeframe than the time taken by collagen pellets with rHuBMP-2 to form ectopic bone. In the ectopic endochondral bone formation process, ossification starts at the border of the cartilage and surrounding tissue.

The results of the RT-PCR analysis of type II collagen and aggrecan revealed that muscle-derived mesenchymal cells differentiated into chondrocytes at 4 days after implantation. However, mature cartilage matrix synthesis started a few days later, since the expression of type IX collagen, which is essential for type II collagen to form cartilage matrix, was weak at 4 days and increased significantly by day 7. Type X collagen and type XI collagen were detected by RT-PCR either with or without rHuBMP-2 in these cells. Because type II collagen and aggrecan were not detected initially, we cannot be sure that chondrogenesis started at the 0 time point. Further work will be needed to map out the exact sequence of expression of these genes in this model. In the absence of rHuBMP-2, the cells expressed type II collagen, but the level was much less when compared with that in cells with rHuBMP-2. This might mean that slow chondrogenesis of muscle-derived mesenchymal cells might occur even in the absence of rHuBMP-2 in this condition.

To examine whether osteogenic differentiation of the muscle-derived mesenchymal cells occurred in this system, we detected Cbfa1/Runx2, which is an essential transcriptional factor for osteoblastic differentiation, by RT-PCR. The expression of Cbfa1/Runx2 was observed at 96 hours in chambers with rHuBMP-2 but not observed in the absence of rHuBMP-2, which means that osteogenic differentiation was initiated by rHuBMP-2. We could not detect the expression of MyoD1 nor were there any cells showing a myogenic phenotype either in the chambers or in the defects at any time point.

The diffusion-chamber–engineered cartilage mass was able to repair full-thickness cartilage defects. At 24 weeks after transplantation, the transplanted cartilage was incorporated and effectively repaired the cartilage defects. The superficial layer of the transplant facing the joint surface had histologic characteristics of articular cartilage, but the greater part beneath the cartilage layer was replaced by bone mass, which was connected to the original subchondral bone. This morphologic condition suggests that part of the transplanted cartilage mass appeared to have features of preossifying cartilage and was in the process of remodeling. This adaptation to the surrounding environment also has been observed in an experiment involving cell transplantation to correct an osteochondral defect (13). When the cartilage plugs that were made in diffusion chambers were implanted into the osteochondral defects, they were replaced by bone from the bone marrow side, but the surface area that was in contact with the joint space remained as cartilage. We believe that the implanted chondrocytes remained at the surface of the defect, although there are no data to support this conclusion.

Adachi et al (21) reported that allogeneic muscle-derived cells embedded in collagen gels are useful for repair of full-thickness articular cartilage, both as a gene delivery vehicle and a cell source for tissue repair. They transduced rabbit allogeneic muscle-derived cells with the β-galactosidase gene (LacZ) and transplanted the cells into the osteochondral defects in the patellar groove in rabbit knees. They reported that the LacZ-positive cells were found in the defect only up to 4 weeks after transplantation. Further studies will be required to more completely understand the biochemical and morphologic processes that underpin the restorative actions of these cell and tissue transplants.

Although the generation of the new cartilage mass and repair of a cartilage defect with the engineered cartilage were shown to be successful in rats, there are some hurdles to be cleared before this approach can be applied in clinical practice. In this study, we used cells from the embryo, which were thought to be more primitive and to have greater capacity for differentiation. However, this represents a problem for clinical application, because of ethical and regulatory issues. Our technique could be applied to muscle-derived cells from the adult, and in this approach, we can use autologous cells. We are planning to apply this system to adult cells, such as bone marrow mesenchymal cells, adipocytes, and muscle-derived cells.

The less responsive nature of muscle-derived mesenchymal cells to rHuBMP-2 in large mammals, including humans, could also be an issue (22). Moreover, the optimal dose of BMP required for cartilage induc-

tion in humans must be determined. In order to solve these issues, further experimental studies in large animals will be essential.

The kinetics of BMP release from collagen is an important consideration. Sellers et al (23) reported that the mean residence time of rHuBMP-2 from a collagen sponge impregnated with 5 μg of rHuBMP-2 was 8 days, with an elimination half-life of 5.6 days. In addition, detectable amounts of rHuBMP-2 were present as long as 14 days after implantation.

In comparing the data from the present study with those reported by Sellers et al, there are differences in experimental details. Sellers et al implanted collagen with 5 μg of rHuBMP-2 into the osteochondral defect, which is likely to result in a rapid vascular invasion and much faster degradation. In the present study, collagen with 10 μg of rHuBMP-2 was placed into the chamber and implanted into subfascial pockets. The presence of the collagen in the chamber impeded invasion by the host cells. In addition, the preparation of a collagen gel and BMP-2 construct were different, and it would be reasonable to expect that the kinetics of BMP release would be influenced by these differences. It is also possible that the transplanted pellets might include BMP-2 at the time of implantation. Consequently, it would be the BMP-2, and not the cells in the pellet, that would drive the regeneration and the stability of repair cartilage. Further studies will be required to understand the kinetics of BMP release and the phenotypic stability of the transplanted cell population, which is believed to play a critical role in the outcome of tissue formation in vivo (24).

The present study has demonstrated another unique application of this approach, namely, the use of muscle-derived mesenchymal cells cultivated in an ex vivo system and differentiation of those cells into chondrogenic cells by rHuBMP-2 in diffusion chambers in an in vivo environment. Use of the muscle-derived mesenchymal cells together with rHuBMP-2 might be a reason for the successful generation of cartilage in this study, because these cells are known to have multilineage differentiation potential (21,25–27). While the present report provides evidence to support this approach for the successful treatment of articular cartilage defects, further studies will be needed to validate the technique for application in clinical practice.

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ORIGINAL ARTICLE

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Potential risk factors for prolonged recovery following whiplash injury

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Abstract A retrospective analysis of insurance data was made of 600 individuals claiming compensation for whiplash following motor vehicle accidents. Three hundred randomly selected claimants who had settled their injury claims within 9 months of the accident were compared with 300 who had settled more than 24 months after the accident. We compared the two groups to identify possible risk factors for prolonged recovery, for which settlement time greater than 24 months was a marker. Variables considered included demographic factors, type of collision, degree of vehicle damage, workers compensation, prior claim or neck disability, treatment and time to settlement. Consulting a solicitor was associated with a highly significant, four-fold increase of late settlement of the claim. A concurrent workers' compensation claim, prior neck disability and undergoing physiotherapy or chiropractic treatment were weakly associated with late settlement. The degree of damage to the vehicle (as indicated by cost of repairs) was not a significant predictor of late settlement. Late settlement may be the direct effect of legal intervention, independent of the severity of the injury. Whilst the financial benefit to the claimant of consulting a solicitor is apparent, the benefit of prolonged disability is not. It may be to the advantage of both insurers and claimants if those likely to proceed to late settlement could be recognised early and their claims settled expeditiously.

Keywords Whiplash · Neck injury · Motor vehicle accident · Compensation claim · Legal representation

Introduction

Whiplash is a common injury. In South Australia approximately 4,000 claims for whiplash, at a cost of the order of Australian $50 million, are made annually (for a population of 1.5 million). There is some evidence that the incidence of this condition has increased in recent decades, although it appears unrelated to increased seat belt use [11].

In an extensive review, the Quebec Task Force (QTF) on whiplash-associated disorders has noted that cases are usually self-limited, with a median time to recovery – measured by time to the end of disability compensation – of 31 days. However, a significant fraction exhibited prolonged disability: 10% of the cases studied in the Quebec cohort study were still unable to resume normal activity 200 days post-injury [8]. In a review of studies published since the QTF report, considerable variation has been found in the duration and extent of recovery. Important sources of variation have been the outcome measures used (e.g., settlement of claim, return to work, persistence of symptoms) and the type of insurance system (e.g., tort or no-fault) [3].

It is unclear if protracted disability from whiplash is related to the degree of trauma. In a 1996 review of whi-

plash, Stovner concluded that evidence for a causal link between trauma and chronic symptoms was sparse [9]. Two studies published since have shown predictive effects of some collision factors (e.g., collisions that are not rear-end) but have failed to show any association between crash severity and prognosis [5, 7].

Unexplained prolonged disability and lack of evidence on effective treatment have led to conflicting opinions on the role of psychological factors and litigation. Some studies in countries with differing insurance-payment systems have found evidence that psychosocial and legal issues may increase morbidity following whiplash injury [4, 6]. However, a randomised double-blinded study in Australia has shown a beneficial effect of radiofrequency neurotomy on chronic whiplash patients. This finding led the authors to propose that psychological effects are likely to be a consequence, rather than the cause, of chronic pain following whiplash and that the most likely cause of pain is post-traumatic dysfunction of the cervical zygapophyseal joints [12]. This study attempts to identify risk factors that may predispose to prolonged disability following whiplash injury.

Materials and methods

Records of whiplash claims filed over the period 1993–1996 were obtained with personal identifiers deleted. This data set was divided into two sub-files: (i) claims settled within 9 months of injury and (ii) claims settled more than 24 months after the injury. Subjects with radiological damage to the cervical spine, neurological deficit and/or significant associated injuries were excluded. Three hundred anonymous records were randomly selected from each sub-file.

A series of univariate analyses was conducted for the relationship between late (>24 months) settlement and the following potential predictors: age, sex, occupation, position in the vehicle, type of collision, prior or concurrent workers' compensation claim, prior neck disability, cost of vehicle repair, whether a solicitor was consulted and cost of treatment. The 600 claims were classified into those with and without the potential risk factor, and the relationship between the risk factors and late settlement was estimated as a risk ratio as follows:

Risk ratio=proportion of subjects with risk factor whose claim was settled after 24 months, divided by the proportion of subjects without the risk factor whose claim was settled after 24 months.

Since one-half of the subjects were selected from the "late settlement" category, the expected proportion of subjects with any risk factor who had a late settlement, in the absence of any association between the factor and late settlement, would be one-half, and the risk ratio equal to one. A risk ratio significantly greater than one would therefore suggest that the risk factor increased the risk of late settlement. The statistical significance of the risk ratio was estimated using a chi-square test. In cases where there were more than two categories of predictor variable (e.g., occupation, mode of injury), a chi-square test for homogeneity was applied to determine whether the distribution differed significantly from the expected value.

A comparison was also made of the distribution of certain variables in the groups in the "early settlement" and "late settlement" categories, using non-parametric analyses. Variables found in the univariate analyses to be significantly related to late settlement were entered into a log binomial model to estimate the role of the variables after adjustment for mutual confounding.

Results

Gender

Of the 600 claimants, 63.5% (381) were female, significantly greater than the proportion of males. Fifty-three percent of the women settled their claims after 24 months, compared with 46% of the men. However, the excess of women with late settlement was not statistically significant (risk ratio=1.15, Table 1).

Age

The proportion of claims settled early for each age stratum is shown in Table 2. In most age strata the proportion of subjects whose settlement was late was close to the expected value of 50%. The exception was subjects aged 65 years and over, of whom only 28% settled late. The latter accounts for the χ^2 value for homogeneity of 13.0, *df* (degrees of freedom)=4, p=0.01. There is no obvious trend away from late settlement for age, and non-parametric testing confirmed that age was not a significant predictor for prolonged settlement.

Occupation

The proportion of late claims by occupation is shown in Table 3. There was no significant association with late settlement in any occupational category (p=0.62).

Type of collision

There was significant variation in the proportion of claims settled late between different types of accident (χ^2=17.3,

Table 1 Settlement time by gender

Sex	Early settlement (<9 months)	Late settlement (>24 months)	Total
Male	119	100	219
Female	181	200	381

Risk ratio for late settlement (F/M)=1.15, NS

Table 2 Settlement time by age

Age range (years)	Early settlement (<9 months)	Late settlement (>24 months)	% with late settlement
0–24	70	61	47
25–44	151	147	49
45–54	36	61	63
55–64	22	23	51
65+	21	8	28

Table 3 Settlement time by occupation

Occupation	Early settlement (<9 months)	Late settlement (>24 months)	% with late settlement
Blue collar	79	70	47
White collar	112	126	53
HD	43	37	46
Unemployed	23	21	48
Pensioner	28	24	46
Student	15	22	59

χ^2=0.62, NS.

Table 4 Settlement time by type of collision

Mode of injury	Early settlement (<9 months)	Late settlement (>24 months)	% with late settlement
Rear hit	146	152	51
Front hit	7	28	80
Rear and front hit	43	39	48
Side hit	92	76	45
Rollover	12	5	29

df=4, p=0.002). Eighty percent of subjects who had experienced a front-end collision had a late settlement. Only 29% of rollovers had a late settlement, although the number of accidents in this category was small. For rear-end, side-impact and chain collisions the proportion of late settlements was close to the expected value of 50% (Table 4).

Position in vehicle

Of the claimants, 423 were drivers, and 177 were passengers, of whom 145 were in the front seat. None of the positions in the vehicle was predictive of early or late settlement of claim.

Workers' compensation

Only 58 of the 600 claims were subject to workers' compensation, of which 46 (79%) had a late settlement, compared with 52% for non-workers' compensation cases. Thus workers' compensation cases were significantly more likely to have a late settlement (risk ratio=1.5, p=0.001). Thirty-five claimants had had a prior workers' compensation claim, but there was no significant association with a history of a prior workers' compensation claim (54% late settlement for those with a previous claim vs 50% with no previous claim). Of employed subjects, the median time off work for those who settled within 9 months was 5 days, compared with 4 days for those who settled late (Kruskal-Wallis χ^2 =0.02, NS).

Table 5 Settlement time by cost of repairs

Cost of repairs	Early settlement (<9 months)	Late settlement (>24 months)	% with late settlement
<$1000	64	54	46
$1000–2500	85	82	49
>$2,500	86	92	52
Written off	65	72	53

Prior neck disability

Of the131 subjects who had a history of neck disability, 58% settled late, compared with 48% for those with no prior neck disability. A history of neck disability was thus predictive of late settlement (risk ratio=1.2, χ^2=4.3, p=0.04).

Damage to vehicle

The cost of repairs as an index of vehicle damage was not a predictor of late settlement. As shown in Table 5, there was no trend towards late settlement with increasing cost of repairs, nor was having the vehicle written off associated with late settlement. Whether the vehicle was driveable after the accident was not a significant predictor of late settlement.

Seeking medical attention on the day of accident

Of the 155 subjects attending a hospital on the day of the accident, 58% settled late compared with 47% of the other subjects. Thus, attending hospital on the day of the accident is a weak but statistically significant predictor of late settlement (risk ratio=1.23, χ^2=5.4, p=0.02). However, a non-hospital medical consultation on the day of the accident had an opposite association. Of the 144 subjects who saw a doctor other than in a hospital on the day of the accident, 41% settled late, compared with 50% of subjects who were not seen at all on the day of the accident, although the effect was not statistically significant (risk ratio=0.69, χ^2=3.3, p=0.07). Overall, attendance at either a hospital or a medical practitioner's rooms on the day of the accident had no association with late settlement (50% late settlement irrespective of attendance).

Attending a physiotherapist or chiropractor

Four hundred seventy subjects attended a physiotherapist some time between the accident and settlement. Those who attended were more likely to settle late (54% vs 32%, risk ratio=1.7, χ^2=20.8, p=0.001); but for these subjects, a risk of late settlement was not associated with the length of time between the accident and first consultation. The median time until the physiotherapist was seen was one

week for both those who settled early and those who settled late. At some time between the accident and settlement, 102 subjects attended a chiropractor. Those who attended were more were likely to settle late (63% vs 47%, risk ratio=1.3, χ^2=8.0, p=0.005). Of the subjects who attended a chiropractor, there was a greater time before the first consultation in those who settled late. The median time until the chiropractor was seen was 1 week in those who settled early and 8 weeks in those who settled late.

Consulting a solicitor

Of the 344 subjects settling their claim through a solicitor, 75% settled late, compared with only 17% of those who settled directly with the insurer. Thus there was a highly significant association between consulting a solicitor and likelihood of a late settlement (risk ratio=14.6, χ^2=197, p=0.001).

Total cost

The median total claim cost for the 300 subjects who settled early was Australian $3,907, and for the 300 who settled late the median cost was $19,457. The difference was significant (p=0.001).

Multivariate analysis

The following variables were entered into a log binomial model: nature of collision (front end, rear end, etc.), making workers' compensation claim, prior neck disability, attending a physiotherapist, attending a chiropractor and consulting a solicitor. As shown in Table 6, there was an elevated risk of late settlement associated with making a worker's compensation claim and prior neck disability. However, the elevation was small in each case (1.15 and 1.14, respectively) and of only marginal statistical significance. Attending either a chiropractor or physiotherapist also accounted for increased risk of late settlement. The increases were small (1.16 and 1.30, respectively) but statistically significant. On the other hand, consulting a solicitor was associated with over a 4-fold increase in risk of late settlement, an increase which was highly significant.

Table 6 Results of multivariate analysis (log binomial model) of possible determinants of late settlement

	Relative risk estimate	95% confidence interval	p-value (χ^2)
Mode of injury	0.98	0.94–1.02	0.33
Workers comp claim	1.15	0.99–1.34	0.08
Prior neck disability	1.14	1.00–1.29	0.06
Attended chiropractor	1.16	1.03–1.29	0.01
Attended physiotherapist	1.30	1.05–1.63	0.02
Consulted solicitor	4.13	3.11–5.48	<0.0001

Discussion

Factors identified as presenting a risk of late settlement were front-end collisions, claims involving workers' compensation, history of prior neck disability, undergoing physiotherapy or chiropractic treatment and consulting a solicitor. By far the strongest association was consultation with a solicitor. The degree of damage to the vehicle (as indicated by cost of repairs) was not a significant predictor. Other factors not predictive of prolonged settlement were a history of prior workers' compensation claim, the period off work, occupational category, whether the subject was the driver or a passenger, and early presentation for medical attention.

The association of front-end collision and late settlement is similar to findings of a recent study of Quebec motor vehicle crashes, in which front and side collisions were found to predict delayed recovery [10]. The Quebec Task Force excludes front-end impact from its definition of whiplash [8]. It is plausible that the distinct dynamics of front-end collisions will yield prognostic markers that differ from those of whiplash injury. However, our multivariate analysis eliminated the nature of the collision as a significant predictor of late settlement: correlation analysis showed that this was not due to collinearity between the type of collision and the other variables.

The association of prior neck disability with late settlement is plausible and consistent with findings from other studies [2]. However, multivariate analysis showed this factor to be only weakly predictive of late settlement. Injury subject to a workers' compensation claim was similarly identified in the initial analysis as predictive of late settlement but found in the multivariate analysis to be only weakly predictive. Correlation analysis showed that this was not due to collinearity with consulting a solicitor (i.e., there was no association between having a work-related motor vehicle injury and consulting a solicitor).

A critical question is whether the duration of disability from whiplash injury is related to the severity of injury. The available data did not provide a direct measure of injury severity. The only available index was the cost of vehicle repair. Since the degree of damage to the vehicle and severity of injury are both related to the amount of energy transfer, some correlation is to be expected. Similarly, a delay in settlement beyond 2 years – the outcome variable used in this study – is not necessarily synonymous with prolonged disability. However, a correlation is likely. Cassidy et al. have reported a strong association between intensity of neck pain and level of physical functioning, on the one hand, and time to closure of the claim for whiplash injury, on the other [1]. Our finding of a lack of as-

sociation between crash severity and prognosis is supported by other recently published studies [5, 7]. On the basis of the indirect measures of both injury severity and duration of disability, these results fail to show any relationship between severity of injury and recovery time.

The strong association between consulting a solicitor and late settlement may be interpreted in two ways: (1) the more severe injury cases may consult a solicitor, with the late settlement resulting from prolonged disability due in turn to the severity of injury; or (2) consultation with the solicitor may be a direct cause of prolonged settlement, independent of the severity of injury. Since our findings, albeit based on indirect measures, showed no association between injury severity and duration of disability, we suggest that the late settlement and increased cost of the claim may be the direct effect of legal intervention and independent of the severity of the injury. Whilst the financial benefit to the claimant of consulting a solicitor is apparent, the benefit of prolonged disability is not. It may be to the advantage of both insurers and claimants if those likely to proceed to late settlement could be recognised early and their claims settled expeditiously.

As factors indicative of greater trauma were not predictive of prolonged settlement, we hypothesise that psychosocial factors are more important determinants of outcome. Accordingly, we are now undertaking a prospective study of whiplash injuries, to measure the influence of psychological, social, physical and emotional well-being on the duration of disability.

As discussed above, there are other dimensions of recovery in addition to settlement of injury claim. These include the return to work, need for continuing treatment and ability to perform activities of daily living. Our prospective study employs a variety of such measures of outcome.

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ENDOSCOPIC SPINAL SURGERY

-RECENT ADVANCES IN THE FIELD OF SPINAL SURGERY

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Endoscopic spinal surgery is one of the recent armamentarium popularized into the medical sciences for the treatment of spine ailments.

Initially in thoracic spine, it was used by the cardiothoracic surgeons for the treatment of lesions in the thoracic cavity. A decreased post-operative morbidity and reduced hospital stay made it increasingly popular amongst the patients as well as the surgeons. Its main indications in thorax include the anterior release for scoliosis, resection of herniated thoracic disc, resection of intrathoracic tumor etc.

In the lumbar spine, it has been used for the excision of the lumbar discs and the fusion of the lumbar spine.

Nowadays, posterior endoscopic disc excision is one of the commonest indications for which this technique is being used.

INTRODUCTION

In recent decades, advances in surgical equipment and refinement of surgical techniques have steadily increased the use of endoscopy in all fields of surgery. These advances are based on the development of optical instruments such as scopes, cameras monitors etc. and have been fostered by the desire of patients to undergo minimally invasive surgery.

In the field of spinal surgery, Obenchain reported the first case of laparoscopic lumbar discectomy, following which Mack et al described video-assisted thoracoscopic (VAT) surgery. Endoscopic surgery has since become one option of surgical treatment for spinal disorders.

THORACOSCOPIC SPINAL SURGERY

In the beginning of the 1990s, thoracoscopic procedures were extensively utilized by cardiothoracic surgeons in the treatment of lesions that involved the thoracic cavity. Following development of equipment and refinement of the technique, the first reports dealing with endoscopic surgery for the thoracic spine were published in early 1990s. Comparisons between thoracoscopy and open thoracotomy have demonstrated that use of

endoscopic techniques decreases postoperative pain, improves shoulder girdle function and decreases morbidity, while reducing blood loss, time required in an ICU and overall length of hospital stay.

The indications for thoracoscopic surgery include biopsy, anterior release for scoliosis, resection of thoracic disc herniation, excision of tumors originating from nerves and reconstruction following vertebrectomy.

ANTERIOR RELEASE FOR SCOLIOSIS

Nearly ten reports have been published on the results of thoracoscopic anterior release for spinal deformity, based on video-assisted thoracoscopic (VAT) surgery and appears best indicated for treatment of moderate curvature (in the range of 55-75 degrees). In a comparative study by Newton et al, VATS technique yielded the same results as open procedures in achieving spinal flexibility, as assessed by average percentage of correction and same results were reported in two further studies. In addition, the rate of complications of the VATS technique was reported to be 18% and almost the same as for the open method.

These preliminary reports enabled correction of scoliosis with newly designed instrumentation under thoracoscopic observation. The operative scar resulting from this surgical procedure is along the midaxillary line. The operative scar resulting from trochar insertion can thus be under the arm, enabling good cosmetic results.

RESECTION OF THORACIC DISC HERNIATION

Resection of thoracic disc herniation is also a good indication for thoracoscopic surgery. Several reports have documented encouraging early results of use of the VATS procedure for thoracic disc herniation. Even over long-term follow-up, endoscopic discectomy yielded results equivalent to those of the classical open technique.

RESECTION OF INTRATHORACIC TUMOR

Resection of para-vertebral tumor in the dumbbell-shaped thoracic cord tumor is also a good indication for thoracoscopic surgery. This type of surgery makes use of a combined posterior and anterior approach. First, a standard posterior approach is used to perform hemilaminectomy of the thoracic spine and of the medial fecetectomy. The intraspinal and foraminal tumor component can usually be resected with a posterior approach. The anterior part of the tumor can then be approached under thoracoscopic observation. Excellent results have thus far been reported with use of this method.

VERTEBRECTOMY, RECONSTRUCTION AND INSTRUMENTATION

Conditions of the anterior column resulting from trauma or infection can be excised, reconstructed or stabilized using anterior instrumentation devices. The beneficial use of the endoscope for such procedures is in the approach to the upper thoracic spine (T2-T4) and the thoracolumbar junction (T11-L2). In the classical open method, the approach to this region is not easy without disinsertion of the scapula or minimal disinsertion of the diaphragm, which necessitates complex reconstructions.

LAPAROSCOPIC LUMBAR SPINAL SURGERY

Laparoscopic discectomy was first described in 1991. This case report of a young male patient with an L5/S1 herniation, in whom surgery was performed by the transperitoneal route with simultaneous endoscopic, video and fluoroscopic guidance, demonstrated the relative ease of access to this disc and in this instance, the procedure could be performed on day care surgery basis. The same group of authors further described their technique

and reported 15 cases of laparoscopic discectomy in 1995. They subsequently switched onto a retroperitoneal approach, which they considered easier and safer, especially at L4/5 and above this level.

Laparoscopic lumbar fusion in humans was first reported in 1995. In that report, complications in 100 endoscopic spinal surgeries including 22 of laparoscopic lumbar fusion were described. The next report concerned a series of six patients, five of whom had successful L5/S1 laparoscopic fusion with bone dowels (metal pins) and in one of whom the endoscopic approach had to be abandoned because of iliac vein laceration. Two further studies reported were of 17 and 34 cases respectively of laparoscopic spinal fusion and each described the approach to the L4/5 as well as the L5/S1-disc.

Recently, due to complications (of laparoscopic discectomy) such as postoperative intra-abnominal adhesions, retrograde ejaculation and great vessel injury, the retroperitoneal endoscopic approach has begun to be utilized. The disadvantage of this approach is the need to retract the psoas muscle, which contains several peripheral nerves. Nakamura et al subsequently described a method of retracting this muscle easily and intermittently. In order to make this approach easier, retroperitoneal endoscopically-assisted mini-laparotomy has been utilized for anterior lumbar interbody fusion. This approach has been reported to have less morbidity than completely closed endoscopic surgery.

POSTERIOR ENDOSCOPIC DISCECTOMY

In 1975, percutaneous lumbar discectomy with posterolateral approach was first reported. Subsequently, this technique evolved to include the use of automated disc removal devices, spinal endoscopy and lasers. However, indications for these procedures have generally been limited to contain lumbar disc herniations, because lumbar radiculopathies (pain in the nerve root) secondary to large free-fragment disc pathology and any type of bony compression of the nerve root are still specific contraindications to percutaneous lumbar discectomy.

In the early 1980s, following the introduction of the technique and instrumentation described by Casper, there was progressive spread of use of microscopes for disc herniation surgery. This has permitted a less invasive approach than the open one, with more rapid postoperative recovery.

Some surgeons have attempted to combine the less invasive microsurgical technique via the traditional midline posterior approach with modern endoscopic technology. Foley and Smith developed a new system for endoscopic posterior discectomy using a tubular retractor. This technique has the same goal as conventional open lumbar discectomy under endoscopic visualization through a small tubular retractor. With this method of true endoscopic surgery, it is possible to successfully remove the disc and/or remove bone lesions compressing a nerve root, as in open approaches, but with a small skin incision and less disruption of the fascia and the paraspinous muscle, reducing postoperative pain. For these reasons, this procedure has greatly decreased the average hospital stay for routine lumbar discectomy.

Since Foley's report, the procedure has been widely utilized for lumbar discectomy. Its relative lack of invasiveness compared with conventional open discectomy has been demonstrated. Recently, resection of recurrent disc herniation and decompression for lumbar spinal canal stenosis has also become an indication for this surgical procedure.

CLINICAL FOCUS

- ★ **Endoscopic spinal surgery is one of the recent advancements and is minimally invasive.**
- ★ **Its main advantages are reduced post-operative morbidity, cosmetically superior results and reduced hospital stay.**
- ★ **Commonly being used for disc surgery and anterior release for scoliosis.**
- ★ **Endoscopic spinal surgery has been recently extended for use in recurrent disc excision and lumbar canal stenosis.**

CONCLUSION

Endoscopic surgery is being commonly used for the treatment of spine problems. It is being used both for thoracic as well as for lumbar spine. Small incisions, short hospital stay and less postoperative pain has led to reduced morbidity to the patients.

The benefits like small scars (cosmetically better) and early return to work are making it more patient-friendly. But a long learning curve and adequate training are mandatory to give satisfactory results.

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ABSTRACTS

MINIMAL ACCESS SPINAL TECHNOLOGIES : STATE-OF-THE-ART, INDICATIONS AND TECHNIQUES

Minimal access spinal technologies aim primarily at minimizing the trauma associated with surgical exposure of the spine. They owe their existence mainly to recent progress in optical and imaging devices and to the development of instrumentations specifically designed for insertion via minimally invasive approaches.

No published scientific studies have proved that minimally invasive techniques are superior over standard techniques. However, patients benefit from the decreased postoperative pain, shorter hospital stay and expedited return to normal activities.

Finally, minimal access spinal technologies are evolving at a fast pace. Progress is being made in defining the indications and assessable results have been obtained for a number of lesions.

SOURCE : Assaker R. et al Neurosurgery Department, Roger Salengro Teaching Hospital, Lille, France. Joint Bone Spine. 2004 Nov;71(6):459-69.

LOW-DOSE ASPIRIN IN GENERAL PRACTICE

Until now, there has been confusing evidence as to whether general practitioners should recommend aspirin to patients to reduce the risk of heart attack and other cardiovascular events. Maria Carla Roncaglioni and collegues used the setting of general practice to carry out a randomised controlled trial to investigate low-dose aspirin in the prevention of cardiovascular events and also looked at a possible role for vitamin E, which is known to prevent oxidative damage.

Low-dose aspirin given by general practitioners in addition to treatment of specific risk factors was found to contribute a beneficial preventive effect. The results for vitamin E, however, were not conclusive. Walter W Rosser discusses the difficulties in carrying out such trials in a general practice setting, but says that general practitioners should now have the confidence to recommend low doses of aspirin for primary prevention. SOURCE :The Lancet, 2001; 357:84, 89.

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Augmentation of bone morphogenetic protein-induced bone mass by local delivery of a prostaglandin E EP4 receptor agonist

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Augmentation of bone morphogenetic protein-induced bone mass by local delivery of a prostaglandin E EP4 receptor agonist

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Abstract

Recombinant human bone morphogenetic protein (rhBMP) is viewed as a therapeutic cytokine because of its ability to induce bone. However, the high doses of rhBMP required for bone induction in humans remain a major hurdle for the therapeutic application of this protein. The development of a methodology that would effectively overcome the weak responsiveness to human BMP is highly desired. In the present study, we investigate the ability of a prostaglandin E EP4 receptor selective agonist (EP4A) to augment the bone-inducing ability of BMP in a biodegradable delivery system. A block copolymer composed of poly-D,L-lactic acid with random insertion of *p*-dioxanone and polyethylene glycol (PLA–DX–PEG, polymer) was used as the delivery system. Polymer discs containing rhBMP-2 and EP4A were implanted into the left dorsal muscle pouch of mice to examine the dose-dependent effects of EP4A. Fifty mice were divided into 5 groups based on the contents of rhBMP and EP4 in the polymer (group 1; BMP 5 μg EP4A 0 μg, group 2; BMP 5 μg EP4 3 μg, group 3; BMP 5 μg EP4 30 μg, group 4; BMP 5 μg EP4 300 μg, group 5; BMP 0 μg EP4 30 μg, $n = 10$ each). All implants were harvested, examined radiologically, and processed for histological analysis 3 weeks after surgery. On dual-energy X-ray absorptiometry (DXA) analysis, the bone mineral content (BMC) of the ossicles was 6.52 ± 0.80 (mg), 9.36 ± 1.89, 14.21 ± 1.27, and 18.75 ± 2.31 in groups 1, 2, 3, and 4 respectively. In terms of BMC, the values of groups 3 and 4 were significantly higher than those of group 1. The mean BMC value of group 4 was approximately 3 times higher than that of group 1. No significant difference in body weight was noted among the groups during the experimental period. In summary, the presence of a prostaglandin E EP4 receptor selective agonist in the carrier polymer enhanced the bone-inducing capacity of rhBMP-2 with no apparent systemic adverse effects.
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Keywords: Bone morphogenetic proteins; Bone metabolism; Bone volume; Bone mineral density; Biomaterials

Introduction

Bone has an inherent regenerating potential, and damaged bone or fractures are repaired by local new bone (callus) formation in a period of several weeks after an injury. The regenerating potential of bone has been attributed to factors or molecules with the biological capacity to induce mesenchymal cells to differentiate into bone- or cartilage-forming cells (osteoblasts and chondrocytes) and thereby form the callus. Bone morphogenetic proteins (BMPs) were originally isolated on the basis of their ability to induce ectopic cartilage and bone formation via an endochondral cascade when implanted in experimental animals [1]. Because of the specific biological activity of BMPs and the successful generation of synthetic BMPs by DNA recombination, there is tremendous interest in using these proteins for bone repair and reconstructive surgery in a clinical setting [2]. However, 2 problems need to be addressed before we can witness the widespread clinical use of rhBMPs. One issue involves the use of a carrier material that has adequate safety and efficacy for BMP delivery. Currently, bovine collagen is used clinically as a carrier for rhBMPs, but use of this material comes with the risk of contracting bovine spongiform encephalopathy (BSE) or Creutzfeldt–Jacob disease (CJD). These diseases are

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potentially transmitted by prion proteins through cattle-derived foods and implant materials. Another problem is the high dose of rhBMP required for clinical efficacy in human patients. For example, to achieve a single level of spinal fusion, several to 10 mg of rhBMP are required. This results in the high cost and limited use of BMP as a substitute for bone autograft. Large doses of BMP may also increase the risk of potential adverse events in patients [3–6].

To address the issue of finding a suitable carrier, we have developed new biodegradable synthetic polymers that work effectively to deliver rhBMP and elicit new bone formation consistently at the implanted sites. The combination of rhBMP-2 and the polymers has enabled the successful regeneration of critical-size bone defects in experimental animals [7–10].

To improve the performance of rhBMP, we have sought agents to reinforce the bone-inducing activity of the protein and increase the induced bone mass. To this end, we have examined phosphodiesterase (PDE) inhibitors (pentoxifylline, rolipram) and a compound (ONO-4819), which is a prostaglandin (PG) EP4 receptor selective agonist (EP4A) [11–13]. PGE produced by cells of the osteoblastic lineage has been implicated as a regulator of bone metabolism through stimulation of either bone formation or resorption [14–16]. Exogenously applied PGE, either systemically or locally, also has enhanced bone formation in in vivo experimental models [17–19]. These biological effects of PGE are mediated through PGE receptors, which have been classified into 4 sub-types, EP1 through EP4. These EP receptors are encoded by distinct genes and are expressed in a tissue-specific manner [20–25]. In general, PGE mediated via EP1 increases intracellular Ca^{2+} concentration, EP2 and EP4 increase cAMP, and EP3 reduces cAMP and modulates down-stream signaling [25]. Knockout mouse studies have revealed that EP4 is the major receptor that mediates the PGE_2-induced anabolic action in bone [26–30]. Systemic administration of an EP4 agonist (ONO-4819) enhanced new bone formation in mice, and an EP4 antagonist suppressed the increase in trabecular bone volume induced by PGE_2 [13,30–33]. In our previous study, the systemic administration of these drugs by daily injection for 1 week during the initial phase of BMP-induced bone formation led to a significant augmentation of ossicle mass [13]. These results suggest that the efficient local release of these activators for BMPs could induce augmented bone formation without adverse effects due to high dose and long-term administration. Therefore, we examined the effects of adding a low dose of ONO-4819 to the BMP delivery system on new bone formation.

Materials and methods

Drugs/chemicals/materials

The prostanoid receptor EP4-selective agonist (ONO-4819), methyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxy-4-(*m*-methoxymethylphenyl)-1-butenyl]-5-oxocyclopentyl]-5-thiaheptanoate (Patent Cooperation Treaty publish No. WO 00/03980), was obtained from Ono Pharmaceutical (Osaka, Japan) and dissolved in phosphate-buffered saline prior to use.

rhBMP-2 was produced by the Genetics Institute (Cambridge, MA) and donated to us through Yamanouchi Pharmaceutical Co. (Tokyo, Japan). The rhBMP-2 was supplied in a buffer solution (5 mmol/l glutamic acid, 2.5% glycine, 0.5% sucrose, and 0.01% Tween-80) at a concentration of 1 μg/μl after filter sterilization.

Poly-D,L-lactic acid–*p*-dioxanone–polyethylene glycol block copolymer (PLA–DX–PEG) (MW; 9800, PLA/DX/PEG molar ratio; LA/Dx/E0 = 43/14/43) was synthesized and provided to us by Taki Chemicals Co. (Kakogawa, Japan). The structural formula of the polymer is shown in Fig. 1. The polymer has a sticky gel-like character at room temperature and turns into a soft gel at 50°C. The physicochemical characteristics and the efficacy of this polymer as a carrier material for rhBMP-2 have been described by our group in previous reports [9,10]. The minimal optimal content of rhBMP-2 required to induce new bone formation was approximately 1 μg in 20 mg of the polymer (0.005%) in mice, 0.02% in rabbits, and 0.04% in dogs based on our previous experimental data [8,10,34].

Animals

One hundred and ten closed colony male ICR mice (4-weeks old; Nippon SLC, Hamamatsu, Japan) were housed and acclimated in cages with free access to food and water for 1 week. Experiments were carried out in strict accordance with the Institutional *Guidelines for the Care and Use of Laboratory Animals* of Osaka City University.

Preparation of PLA–DX–PEG polymer implants containing rhBMP-2 and ONO-4819

To prepare a single implant, 30 mg of the PLA–DX–PEG polymer was softened by heating to 37°C, mixed with an aliquot of either the rhBMP-2 solution (0.5 μg/5

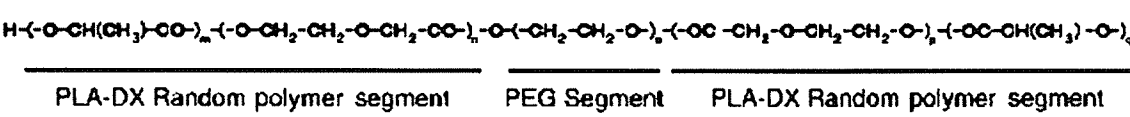

Fig. 1. Structural formula of PLA–DX–PEG polymer. Structural formula of the poly-D, L-lactic acid with random insertion of *p*-dioxanone and polyethylene glycol block copolymer (PLA–DX–PEG). The subscripts m, n, o, p, and q represent variable numbers of these units.