Tissue engineering of the intervertebral disc with cultured annulus fibrosus cells using atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS scaffold)

M. Sato[1,2] M. Kikuchi[2] M. Ishihara[2] M. Ishihara[3] T. Asazuma[4] T. Kikuchi[4] K. Masuoka[4] H. Hattori[3] K. Fujikawa[4]

[1]Department of Orthopedic Surgery, Japan Self-Defense Force Yokosuka Hospital, Yokosuka, Kanagawa, Japan
[2]Department of Medical Engineering, National Defense Medical College, Tokorozawa, Saitama, Japan
[3]Division of Biomedical Engineering, Research Institute, National Defense Medical College, Tokorozawa, Saitama, Japan
[4]Department of Orthopaedic Surgery, National Defense Medical College, Tokorozawa, Saitama, Japan

Abstract—*The objective of the study was to investigate the regeneration of intervertebral discs after laser discectomy using tissue engineering procedures. Annulus fibrosus (AF) cells from the intervertebral discs of Japanese white rabbits were cultured in an atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS scaffold), to produce a high-density, three-dimensional culture for up to 3 weeks. Although the DNA content in the scaffold increased at a lower rate than that in the monolayer culture, expression of type II collagen and glycosaminoglycan accumulation in the scaffold were at higher levels than in the monolayer. The AF cells that had been cultured in the scaffold for 7 days were allografted into the lacunae of intervertebral discs of recipients (40 rabbits, 14–16 weeks old; average weight, 3.2 kg), whose nucleus pulposus (NP) had been vaporised with an ICG dye-enhanced laser. The allografted cultured AF cells survived and produced hyaline-like cartilage. Furthermore, the narrowing of the intervertebral disc space of the cell-containing scaffold insertion groups was significantly inhibited after 12 post-operative weeks.*

Keywords—*Intervertebral disc, Annulus fibrosus, Regeneration, Tissue engineering, Cultured cells*

Med. Biol. Eng. Comput., May 2003, **41**, 365–371

1 Introduction

THE INTERVERTEBRAL disc, like articular cartilage, is the most avascular tissue in the human body, and its capacity for regeneration is as low as that of articular cartilage (POIRAUDEAU *et al.*, 1999). The disc has two distinct anatomic regions: the annulus fibrosus (AF) is the fibrous ring forming the circumference of the intervertebral disc; and the nucleus pulposus (NP) is the remnant of the notochord forming the central portion of the intervertebral disc. Each region contains an abundant extracellular matrix surrounding cells with different morphologies (CHIBA *et al.*, 1997; POIRAUDEAU *et al.*, 1999). Except for the large amount of type I collagen in the layers of parallel bundles of dense connective tissue, most of the intervertebral matrix molecules are cartilage-type (large aggregates of proteoglycans and type II collagen) (GRUBER *et al.*, 1997). In fact, AF cells mainly express cartilage-type matrix molecules, which have quantitative differences as a function of cell morphology (GRUBER *et al.*, 1997; POIRAUDEAU *et al.*, 1999).

When nucleotomy, a surgical treatment for intervertebral disc herniation, is performed, there is little regeneration of either the NP or the AF. Although treated patients usually obtain relief from sciatica after such procedures, post-operative acceleration of disc degeneration is often observed and ultimately can lead to a loss of mobility, as well as persistent low back pain (CHIBA *et al.*, 1997; NISHIMURA and MOCHIDA 1998; GAN *et al.*, 2000).

We have attempted a non-invasive surgical procedure, percutaneous laser disc decompression (PLDD) using an ICG dye-enhanced laser, as its high selectivity enables safe vaporisation of the NP without causing major damage to the surrounding tissues (SATO *et al.*, 2001*a*). Such selectivity is a significant advantage

Correspondence should be addressed to Dr Masato Sato; email: satomd@cc.ndmc.ac.jp

Paper received 23 July 2002 and in final form 29 January 2003
MBEC online number: 20033778

over PLDD performed with other lasers. Nevertheless, intervertebral disc herniation involves degeneration of the AF, and even minimally invasive surgical procedures cause additional damage to the AF as well as NP tissues, hastening further degeneration. Thus natural healing is still required if adequate repair of the intervertebral disc tissue is to occur.

MOCHIDA *et al.* (1996) reported that the progress of intervertebral disc degeneration following percutaneous nucleotomy is dependent on the amount of NP extracted. Transplantations of cultured articular chondrocytes by PETERSON *et al.* (2000) and of cultured chondrocytes within aterocollagen gel by OCHI *et al.* (2001) are already in clinical trials for the repair and regeneration of articular cartilage. Although tissue engineering concepts have thus been applied for the repair of articular cartilage using chondrocyte-seeded implants, to the best of our knowledge no study has been reported of the application of a scaffold seeded with AF cells to prevent intravertebral disc degeneration.

A new atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS scaffold), which allows high-density, three-dimensional culture of various cells, has been reported (ITOH *et al.*, 2001) as a tissue engineering scaffold. In the present study, the ACHMS scaffold has been evaluated for *in vitro* use in the three-dimensional culture of AF cells. We also allografted the three-dimensional, cultured AF cells in the scaffold into the lacunae of intervertebral discs whose NP had been vaporised with an ICG dye-enhanced laser. Subsequent regeneration of the intervertebral disc was assessed on the basis of the viability and histological status of the allografted, cultured AF cells.

2 Materials and methods

2.1 *Preparation of atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS scaffold)*

Preparation of the atelocollagen honeycombs has been described previously (ITOH *et al.*, 2001)*. Briefly, type I atelocollagen in solution (1%, pH 3) was poured into a shallow tray and exposed to ammonia gas to neutralise it. After the excessive ammonia had been washed out with distilled water, the white gel was freeze-dried, and the resulting honeycomb-shaped sponge was sliced. The 2 mm thick slices were irradiated by ultraviolet (UV) light to yield an insoluble honeycomb-shaped scaffold (ITOH *et al.*, 2001).

To prepare the collagen-membrane seal, type I atelocollagen solution (1%, pH 3) was poured into the tray and exposed to ammonia gas to neutralise it. The honeycomb-shaped scaffolds were placed on the thin gel produced and air-dried on a clean bench. After making the membrane insoluble by UV irradiation, the ACHMS scaffolds were cut into round shapes using a punch. Finally, the round ACHMS scaffolds were treated at 130°C for 20 min in a vacuum for sterilisation (Figs 1*a* and *b*).

2.2 *Culturing of annulus fibrosus cells from intervertebral disc*

The AF from the intervertebral discs of Japanese white rabbits (4 weeks old; average weight, 1.0 kg) was shredded and then digested in F12/DMEM† containing 0.4% (w/v) pronase E‡ for 1 h, and then in F12/DMEM** containing 0.025% (w/v) bacterial collagenase P†† for 4 h (SATO *et al.*, 2001*b*). The digested tissue was passed through a cell strainer with a pore size of 40 μm‡‡, and the filtrate was centrifuged at 400 *g* for 10 min to separate the cells. The cells (0.5 ml) were then seeded at a high density (2×10^6 cells per scaffold) into the round ACHMS scaffold (diameter: 11 mm; thickness: 2 mm) in 48 well plates*, by being centrifuged at 40 *g* for 5 min, and cultured in F12/DMEM supplemented with 10% fetal bovine serum** and 50 $\mu g/ml^{-1}$ ascorbic acid† at 37°C in an atmosphere of 5% CO_2 in air and 100% relative humidity. The AF cells were also plated at a density of 2×10^6 cells per dish into culture dishes‡ (diameter: 10 cm) and cultured as a monolayer culture under the same conditions as described above.

2.3 *Scanning electron microscopy*

Samples were fixed in 2.5% glutaraldehyde** and 1% osmium acid**. After dehydration through a graded series of water-ethanol and ethanol-isoamyl acetate solutions, the samples were critical point-dried††. Samples were cut with a sharp knife so that the intra-scaffold could be observed and subsequently shadowed with gold using an ionspatter‡‡. They were then observed with a scanning electron microscope[1].

2.4 *Fluorimetric assay of DNA*

The DNA contents in the papain digests of the cells were determined by a fluorimetric assay (KIM *et al.*, 1988; SATO *et al.*, 2001*b*). Briefly, 15 μl of the papain digest was mixed with 300 μl of Hoechst 33258[2] solution in a 96-well fluoroplate. Emission and excitation spectra were obtained with a Titertek multiscan spectrofluorometer[3] at 456 nm and 365 nm, respectively. DNA concentrations were determined against a standard curve of calf thymus DNA[4] ranging from 0.625 to 40 $\mu g/ml^{-1}$.

2.5 *Western blotting analysis of type II collagen*

Type II collagen was extracted from both monolayer-cultured AF cells and AF cells within the scaffold on day 21. SDS polyacrylamide gel electrophoresis of the collagen preparations was carried out, and the gel was stained with Coomassie blue, as previously described (BURGESON and HOLLISTER, 1979). Western blotting analysis for type II collagen was performed according to the method of KUMAGAI *et al.* (1994). Briefly, type II collagen was separated on an SDS polyacrylamide gel (35 min, 200 V), and the protein bands were electroblotted on a nitrocellulose membrane and reacted with an anti-collagen type II monoclonal antibody[5]. Immunoreaction was visualised using the avidin-biotinylated horseradish peroxidase (HRP) complex method.

2.6 *Assay to determine the glycosaminoglycan content*

To examine the glycosaminoglycan (GAG) content in AF cells, the cell-matrix component was assayed using the 1,9-dimethylmethylene blue (DMB) method described by FARNDALE *et al.* (1986). Briefly, the cell cultures within the honeycomb-shaped scaffold on days 2, 5, 10 and 14 were digested with 300 $\mu g/ml^{-1}$ papain in 50 mM phosphate buffer, pH 6.5, containing 2 mM N-acetyl cysteine and 2 mM EDTA at 60°C, overnight. The cells cultured in a monolayer were also harvested using a scraper and digested with papain as described

*Performed by Koken Inc., Tokyo, Japan
†Nissui Pharmaceutical, Tokyo, Japan
‡Kaken Pharmaceutical, Tokyo, Japan
**Iwaki, Tokyo, Japan
††Boehringer Mannheim, Mannheim, Germany
‡‡Becton Dikinson, NJ, USA

*Sumitomo Bakelite, Tokyo, Japan
†Sigma, St Louis, MO, USA
‡Falcon, Nippon Becton Dickinson, Tokyo, Japan
**Wako Pure Chemical, Osaka, Japan
††HCP-2, Hitachi, Tokyo, Japan
‡‡JFC1100, Nihon Denshi Corp., Tokyo, Japan
[1]JSM-840, Nihon Denshi Corp., Tokyo Japan
[2]Polyscience, Warrington, PA, USA
[3]Lab Systems, Helsinki, Finland
[4]Sigma, St Louis, MO, USA
[5]II-4CII, Fuji, Chem. Ind., Tokyo, Japan

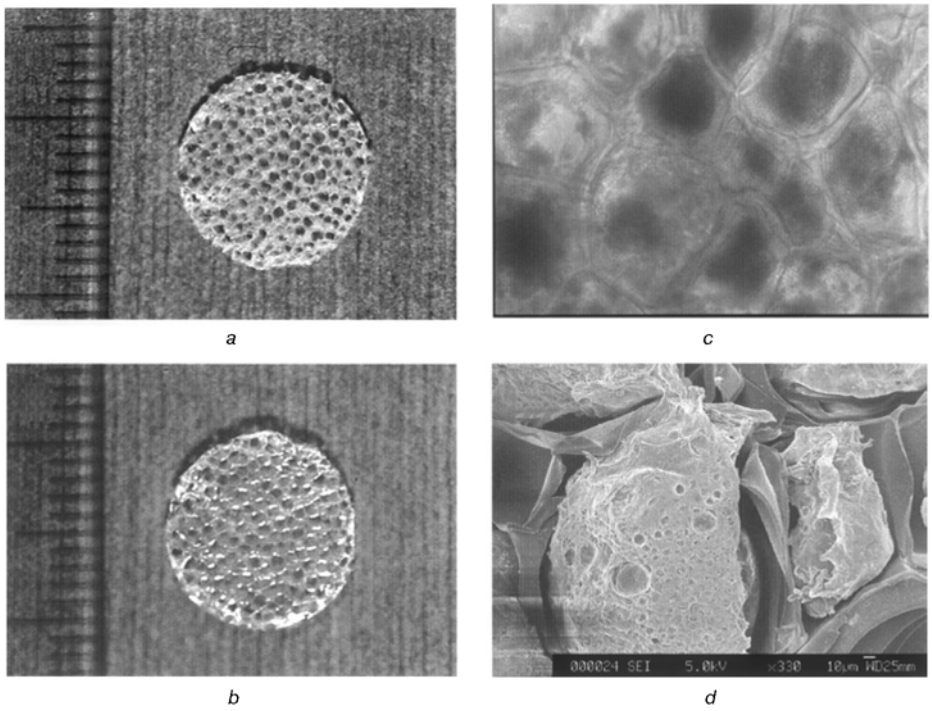

Fig. 1 *Appearance of honeycomb-shaped atelocollagen scaffold with membrane seal (ACHMS scaffold), and microphotographs of AF cell-seeded scaffolds. (a) top of scaffold; (b) bottom of scaffold with membrane seal; (c) phase-contrast microphotograph of scaffold after plating 2×10^6 AF cells per scaffold (original magnification; ×100); (d) scanning electron microphotograph of scaffold-cultured AF cells after 21 days (original magnification; ×330)*

above. The sample solutions (140 µl) were gently mixed with an equal volume of DMB solution in a 96-well microtiter plate, and the absorbance at 530 nm was measured immediately using the Titertec multiscan spectrophotometer. Absorbance values were quantified against a standard curve of shark chondroitin sulphate C[‡‡], ranging from 0.625 to 20 µg/ml^{-1}.

2.7 *Laser irradiation and transplantation*

Forty female Japanese white rabbits (14–16 weeks old; average weight, 3.2 kg) were classified into the following groups:

(i) a cell-containing scaffold insertion group, in which AF cells cultured in an ACHMS scaffold were allografted after laser vaporisation
(ii) a scaffold insertion group, in which only the ACHMS scaffold was inserted after laser vaporisation
(iii) a non-insertion group, in which laser vaporisation was performed, but neither cells nor the scaffold were inserted
(iv) a sham group, in which a sham operation (without vaporisation or insertion) was carried out.

The rabbits were anaesthetised with intramuscular injections of 120 mg ketamine and 9 mg xylazine. A vertical incision (about 8 cm long) was made dorsally, and subcutaneous ablation revealed the area from the peritoneum and lateral musculus rectus abdominus to the intervertebral discs. A 27-gauge needle was inserted into three intervertebral discs (L3/4–L5/6), and a micro-injector was used to inject 20 µl of ICG* solution (1 mg/ml^{-1}), which had an absorption peak of 805 nm in the absorption spectrum of a diode laser[†] (power output: 25 W). A hole (diameter: 2.5 mm) was made that extended through the AF to the NP (see Fig. 5 later). Subsequently, diode laser intermittent irradiation for 15 s, through a single silica fibre[‡] 600 µm in diameter, delivered a total energy of 50 J to vaporise the NP. The ACHMS scaffold, containing 1-week cultured AF cells, was cut longitudinally into four sections that were inserted using a cannula consisting of a 16-gauge Surflo catheter[**] into the lacunae after vaporisation of the NP, as well as into the hole in the AF (see Fig. 5).

2.8 *Evaluation of disc space and histological evaluation*

Soft X-ray photographs of the lumbar spine of the anaesthetised rabbits were taken with a super-soft X-ray apparatus[††]. The disc space of two rabbits from each of the four recipient groups was measured using the three identical discs (L3/4, 4/5, 5/6) from each these rabbits ($n = 6$, total of 24 discs). Progressive decreases in intervertebral disc space were found at 2, 8 and 12 post-operative weeks. The narrowing of the disc space was determined using the Bradner disc index (BRADNER *et al.*, 1972; SUMIDA *et al.*, 1999). Each value was compared with the pre-operative value.

Consecutive frozen cross-sections about 6 µm thick were prepared from the intervertebral discs of recipients, and the cartilage matrix of the samples was stained with safranin-O[‡‡] for histological evaluation.

2.9 *Statistical analysis*

In the statistical analysis of the results, the Dunn procedure was performed as a multiple comparison after one-way ANOVA had been evaluated among all combinations.

3 Results

3.1 *Cell proliferation in the ACHMS scaffold*

A density of AF cells of 2×10^6 cells per scaffold could be seeded into the ACHMS scaffold by gentle centrifugation without spilling cells, owing to the scaffold membrane seal (see Fig. 1*c*).

‡‡Seikagaku Kogyo, Tokyo, Japan
*Diagnogreen, Daiichi pharmaceutical, Tokyo, Japan
†Diomed 25, Diomed, Cambridge, UK
‡DM-6060, Olympus, Tokyo, Japan

**Terumo Co. Ltd., Tokyo, Japan
††Softex-CSM-2, Softex, Osaka, Japan
‡‡Merck Co. Ltd., Germany

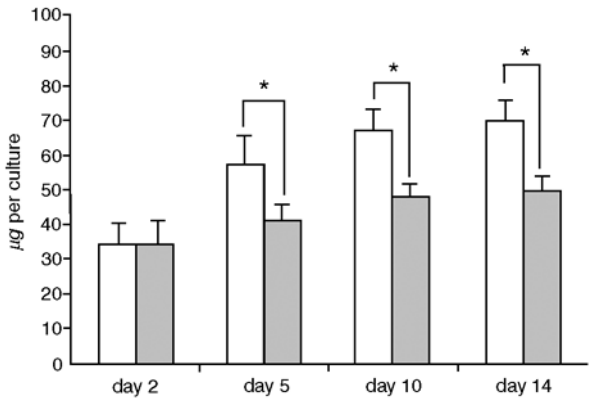

Fig. 2 *DNA content in scaffold AF cell cultures using (□) monolayer and (■) scaffold cultures. Measurement of DNA content was conducted on indicated days, as described in Section 2. Data represent means ± SD of tetraplicate determinations*

The DNA content in the scaffold culture of AF cells increased at a slower rate when compared with the monolayer culture over 14 days (Fig. 2). However, microscopic observations revealed that, on day 21, the scaffold was filled with grown AF cells surrounded by matrix molecules they had produced. It was also found that AF cells in the scaffold retained their spherical shape and typical chondrocytic appearance during the culture period. Furthermore, it was observed that cartilage lacunae were formed around the cells (Fig. 1*d*). Thus AF cells in the scaffold were able to survive and grow gradually for a longer period of time (at least 3 weeks), having a lower growth rate than in a monolayer culture and producing matrix molecules in the scaffold culture.

3.2 *Western blotting of type II collagen and GAG accumulation*

Western blotting of type II collagen showed that the accumulation of type II collagen within the scaffold culture was much higher than in the monolayer culture (Fig. 3). The increase rate of the total GAG content from the cell-matrix component of the scaffold culture was also higher than that of the monolayer culture from an early stage (day 2), and this difference was observed throughout the entire culture period up to 14 days (Fig. 4). Thus, AF cells in the scaffold were able to produce and accumulate significantly higher amounts of extracellular matrix molecules than those in the monolayer culture.

3.3 *Changes in disc space* in vivo

Fig. 5 shows the experimental schemes of the inhibitory effect of the AF cell-containing scaffold insertion for narrowing the intervertebral disc space. Progressive narrowing of the intervertebral disc space was observed to different extents in the cell-containing scaffold insertion group, the scaffold insertion group and the non-insertion group (Fig. 6). There was a greater degree of narrowing in the non-insertion group than in the scaffold insertion group and the cell-containing scaffold insertion group at post-operative week 2. At 12 post-operative weeks, the non-insertion group and the scaffold insertion group showed narrowing of 33% and 45%, respectively, whereas the narrowing of the cell-containing scaffold insertion group was still less than 20%, representing a significant difference ($p < 0.05$, Fig. 6).

3.4 *Histological findings*

In the non-insertion group, a minor cellularity was generated upon the laser vaporisation, and the amorphous tissue was stained weakly with safranin-O in the AF and NP (Figs 7*a* and 8*a*). In the AF and NP of the scaffold insertion group, the scaffold was surrounded by cicatricial tissue with a minor cellularity and weak staining (Figs 7*b* and 8*b*). In the AF and NP of the cell-containing scaffold insertion group, a marked accumulation of cartilage-like matrix with strong staining was observed inside and around the scaffold, forming hyaline-like cartilage with strong safranin-O staining (Figs 7*c* and 8*c*). These were histologically similar to normal AF and NP tissues (Figs 7*d* and 8*d*).

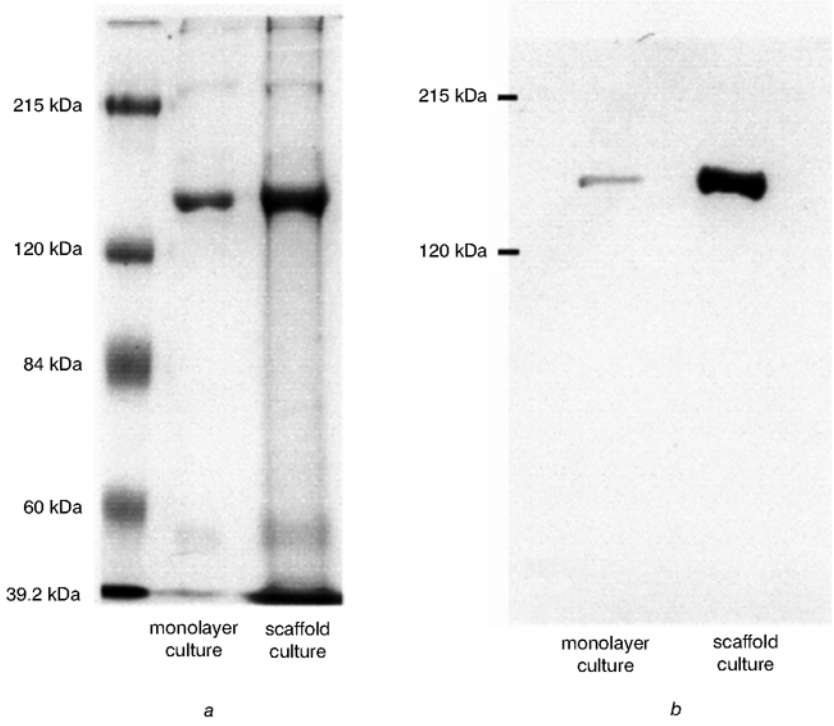

Fig. 3 *Western blotting of type II collagen. Type II collagen was extracted from monolayer and scaffold AF cell cultures on day 21, and (a) SDS polyacrylamide gel electrophoresis for collagen extractions and (b) Western blotting analysis of type II collagen were carried out, as described in Section 2*

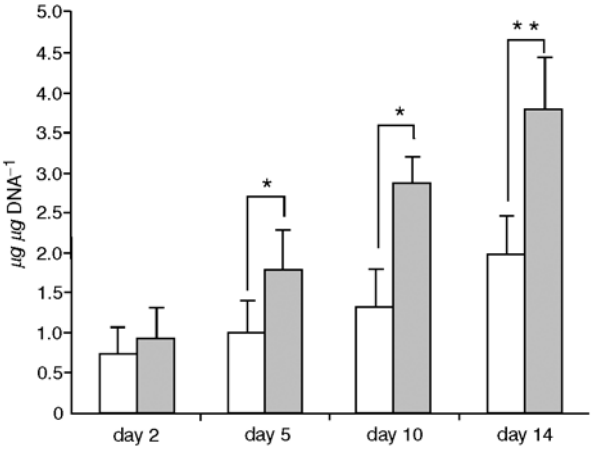

Fig. 4 *GAG accumulation in (□) monolayer and (■) scaffold AF cultures, measured on indicated days. Data represent means ± SD of tetraplicate determinations*

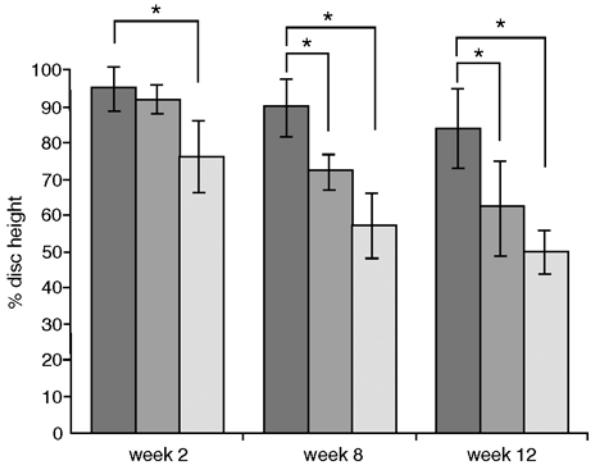

Fig. 6 *Progressive narrowing of intervertebral disc space. Narrowing of disc space was smaller in (■) cell-containing scaffold insertion group than in ( ) non-insertion group and (■) scaffold insertion group (*$p < 0.05$, against cell-containing scaffold insertion group, $n = 6$ for each group). Disc height (Bradner's index) of sham-operated animals was defined as 100%. Data represent means ± SD ($n = 6$)*

4 Discussion

The usefulness of an atelocollagen honeycomb-shaped scaffold in the *in vitro* mass culture of various cells has been reported (ITOH *et al.*, 2001; SATO *et al.*, 2002). Although the growth of AF cells in the ACHMS scaffold appeared to be slower than in the monolayer culture in the present study, the scaffold culture maintained viability and the ability to produce type II collagen and proteoglycans. The syntheses of type II collagen and proteoglycans have been considered sensitive metabolic markers with which to investigate the phenotype level in studies dealing with chondrocytes isolated from hyaline cartilage tissue (FRENKEL *et al.*, 1997; LOTY *et al.*, 1998) and AF cells from intervertebral discs (CHIBA *et al.*, 1997; POIRAUDEAU *et al.*, 1999). Thus the present *in vitro* results indicate the usefulness of this scaffold as a biomaterial for tissue engineering of intervertebral discs using AF cells.

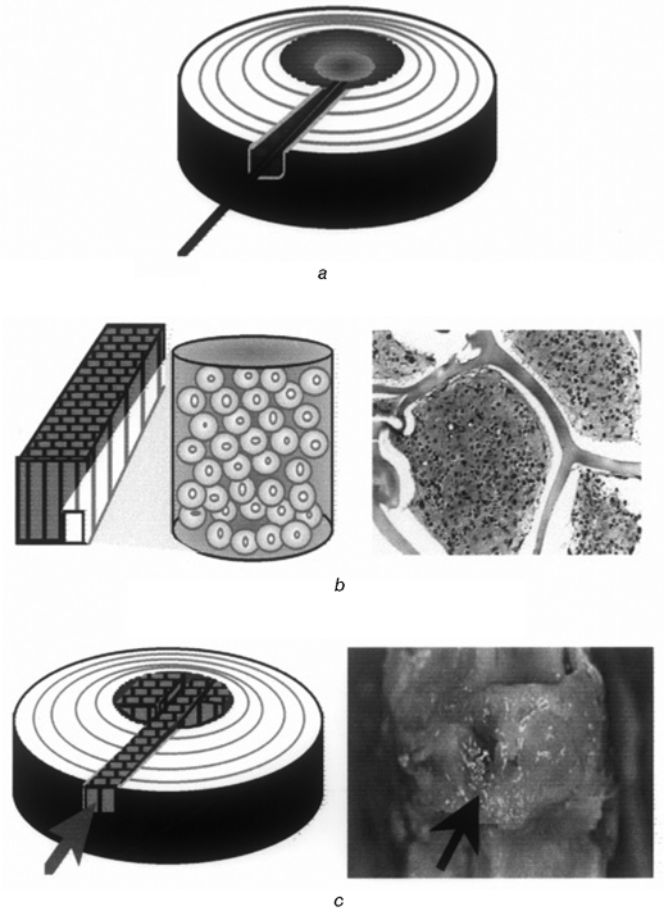

Fig. 5 *In vivo experimental scheme. (a) Laser discectomy with ICG-enhanced diode laser. (b) Three-dimensional cultured AF cells in ACHMS scaffold: (c) Allograft of tissue-engineered tissue into lacunae of intervertebral disc*

The intervertebral disc has a high degree of heterogeneity: it contains both the NP and the AF, as well as having an irregular cell distribution. The NP contains a large volume of water, and its matrix is mechanically weak. The NP cells of young rabbits are mainly large vacuolated cells with a small number of smaller cells (POIRAUDEAU *et al.*, 1999). On the other hand, AF cells are chondrocyte-like cells within a dense, mechanically strong matrix (POIRAUDEAU *et al.*, 1999). In a rat model in which herniations were induced in the caudal vertebrae of rats (NISHIMURA and MOCHIDA, 1998), the reinsertion of either fresh or cryopreserved NP was found to prevent the progression of intervertebral disc degeneration. Okuma *et al.* reported similar success in controlling the progress of intervertebral disc degeneration in rabbit by reinsertion of the NP (OKUMA *et al.*, 2000). In addition, Nishida *et al.* reported the possibility of treating degenerating intervertebral discs by introducing the TGF-β1 gene into NP cells of rabbits using an adenovirus as a vector (NISHIDA *et al.*, 1998). However, intervertebral disc herniation also involves degeneration of the AF, and even minimally invasive surgical procedures cause additional damage to the AF, accelerating further degeneration.

The goal of regenerating the intervertebral disc tissue is not only to achieve anatomic morphology, but also to restore disc function. Both the NP and AF tissues should first be regenerated in order for both histological and mechanical functions to be regenerated. In the present study, we evaluated *in vivo* intervertebral disc regeneration with AF cell-containing ACHMS scaffold using the rabbit intervertebral disc degeneration model. The allografted AF cells exhibited proliferation activity, resulting in the production of hyaline-like cartilage, and the regenerated AF tissue was found to be histologically similar to the original. The mechanical functioning of regenerated intervertebral discs, especially for NP tissue, remains to be investigated. However, the present finding, that the narrowing of the intervertebral disc space was significantly prevented in the cell-containing scaffold insertion group, suggests that both

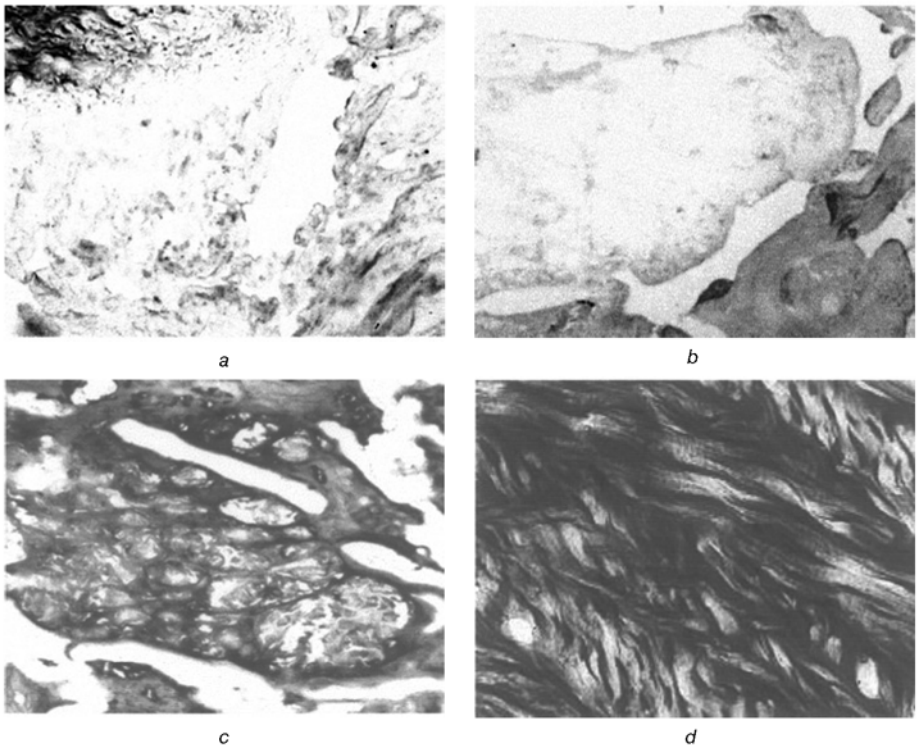

Fig. 7 *Observations of allografted AF cells in AF tissues in intervertebral disc 12 weeks after allograft: (a) No staining in damaged AF of non-insertion group (safranin-O staining, ×100). (b) Carrier with minor cellularity in AF of scaffold insertion group (safranin-O staining, ×100). (c) Allografted AF cells and accumulated matrix intensely stained in AF of cell-containing scaffold insertion group (safranin-O staining, ×100). (d) Normal tissue in AF of sham group (safranin-O staining, ×100)*

suppression of intervertebral disc degeneration and improvement of mechanical strength can be achieved.

In summary, we used tissue-engineering methods to carry out allografting to regenerate AF and NP cells damaged by laser vaporisation. The allografted AF cells exhibited proliferation activity and production of extracellular matrix, both *in vitro* and *in vivo*, and prevented the narrowing of the intervertebral disc space *in vivo*. These results suggest that it may be possible to

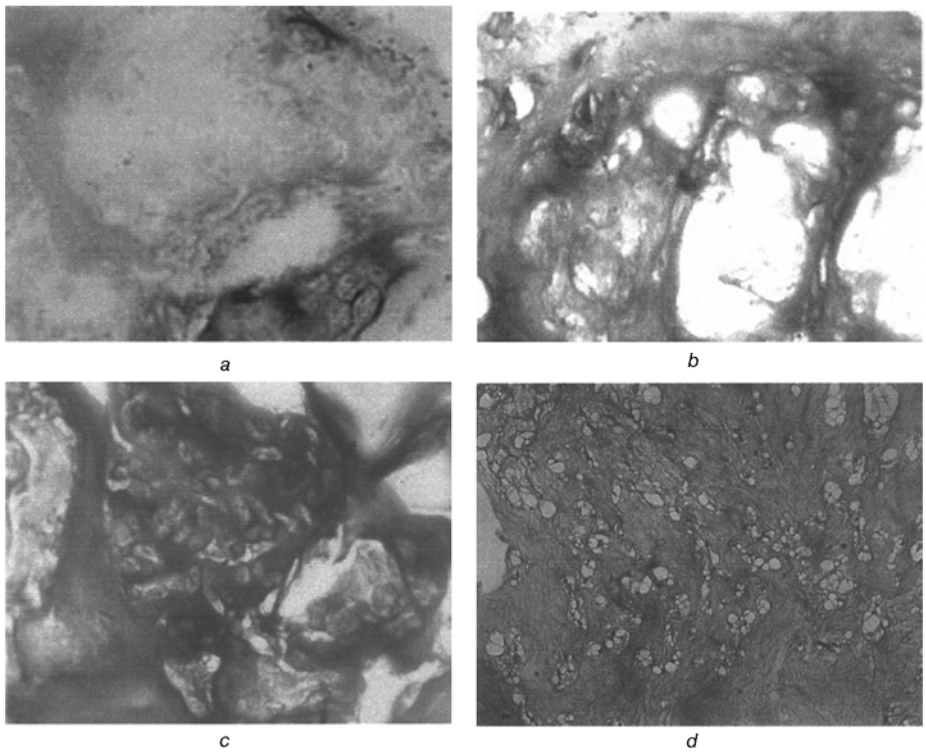

Fig. 8 *Observations of allografted AF cells in NP tissues in intervertebral disc 12 weeks after allograft: (a) No staining in damaged NP of non-insertion group (safranin-O staining, ×100). (b) Carrier with minor cellularity in NP of scaffold insertion group (safranin-O staining, ×100). (c) Allografted AF cells and accumulated matrix intensely stained in NP of cell-containing scaffold insertion group (safranin-O staining, ×100). (d) Normal tissue in NP of sham group (safranin-O staining, ×100)*

achieve regeneration of intervertebral discs through allografting of AF cells in an ACHMS scaffold.

References

BRADNER, M. E. (1972): 'Normal values of the vertebral body and intervertebral disc index in adults', *Am. J. Rentgenol. Radium Ther. Nucl. Med.*, **114**, pp. 411–414

BURGESON, R. E., and HOLLISTER, D. W. (1979): 'Collagen heterogeneity in human cartilage: identification of several new collagen chains', *B B R C*, **87**, pp. 1124–1131

CHIBA, K., ANDERSON, G. B. J., MASUDA, K., and THONAR, E. J. (1997): 'Metabolism of the extracellular matrix formed by intervertebral disc cells cultured in alginate', *Spine*, **22**, pp. 2885–2893

FARNDALE, R. W., BUTTLE, A. J., and BARRETT, A. J. (1986): 'Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue', *Biochem. Biophys. Acta*, **883**, pp. 173–177

FRENKEL, S. R., TOOLAN, B., MENCHE, D., PITMAN, M. I., and PACHENCE, J. M. (1997): 'Chondrocyte transplantation using collagen bilayer matrix for cartilage repair', *J. Bone Joint Surg.*, **79-B**, pp. 831–836

GAN, J. C., DUCHEYNE, P., VRESILOVIC, E., and SHAPIRO, I. M. (2000): 'Bioactive glass serves as a substrate for maintenance of phenotype of nucleus pulposus cells of intervertebral disc', *J. Biomed. Mater. Res.*, **51**, pp. 596–604

GRUBER, H. E., FISHER, E. C., DESAI, B., STASKY, A. A., HOELSCHER, G., and HANLEY, E. N. (1997): 'Human intervertebral disc cells from the annulus: three-dimensional culture in agarose or alginate and responsiveness to TGF-β1', *Exp. Cell Res.*, **235**, pp. 13–21

ITOH, H., ASO, Y., FURUSE, M., NOISHIKI, Y., and MIYATA, T. (2001): 'A honeycomb collagen carrier for cell culture as a tissue engineering scaffold', *Artif. Organs*, **25**, pp. 231–237

KIM, Y. J., SAH, R. L., DOONG, J. Y., and GRODZINSKY, A. J. (1988): 'Fluorometric assay of DNA in cartilage explants using Hoechst 33258', *Anal. Biochem.*, **174**, pp. 168–176

KUMAGAI, J., SARKAR, K., UHTHOFF, H. K., OKAWARA, Y., and OOSHIMA, A. (1994): 'Immunohistochemical distribution of type I, II, and III collagens in the rabbit supraspinatus tendon insertion', *J. Anat.*, **185**, pp. 279–284

LOTY, S., SAUTIER, J-M., LOTY, C., BOULEKBACHE, H., and KOKUBO, T. (1998): 'Cartilage formation by fetal rat chondrocytes cultured in alginate beads: a proposed model for investigating tissue-biomaterial interactions', *J. Biomed. Mater. Res.*, **42**, pp. 213–222

MOCHIDA, J., NISHIMURA, K., NOMURA, T., TOH, E., and CHIBA, M. (1996): 'The importance of preserving disc structure in surgical approaches to lumbar disc herniation', *Spine*, **21**, pp. 1556–1563

NISHIDA, K., KANG, J. D., SUH, J. K., ROBBINS, P. D., EVANS, C. H., and GILBERTSON, L. G. (1998): 'Adenovirus-mediated gene transfer to nucleus pulposus cell: implications for the treatment of intervertebral disc degeneration', *Spine*, **23**, pp. 2437–2442

NISHIMURA, K., and MOCHIDA, J. (1998): 'Percutaneous reinsertion of nucleus pulposus: an experimental study', *Spine*, **23**, pp. 1531–1539

OCHI, M., UCHIO, Y., TOBITA, M., and KURIWAKA, M. (2001): 'Current concepts in tissue engineering technique for repair of cartilage defect', *Artif. Organs*, **25**, pp. 172–179

OKUMA, M., MOCHIDA, J., NISHIMURA, K., SAKABE, K., and SEIKI, K. (2000): 'Reinsertion of stimulated nucleus pulposus cells retards intervertebral disc degeneration: an *in vitro* and *in vivo* experimental study', *J. Orthop. Res.*, **18**, pp. 988–997

PETERSON, L., MINAS, T., BRITTBERG, M., NILSSON, A., SJOGREN-JANSSON, E., and LINDAHL, A. (2000): 'Two- to 9-year outcome after autologous chondrocyte transplantation of the knee', *Clin. Orthop.*, **374**, pp. 212–234

POIRAUDEAU, S., MONTEIRO, I., ANRACT, P., BLANCHARD, O., REVEL, M., and CORVOL, T. (1999): 'Phenotypic characteristics of rabbit intervertebral disc cells: comparison with cartilage cells from the same animals', *Spine*, **24**, pp. 837–844

SATO, M., ISHIHARA, M., ARAI, T., ASAZUMA, T., KIKUCHI, T., HAYASHI, T., YAMADA, T., KIKUCHI, M., and FUJIKAWA, K. (2001a): 'Use of a new ICG-dye-enhanced diode laser for percutaneous laser disc decompression', *Lasers Surg. Med.*, **29**, pp. 282–287

SATO, M., KIKUCHI, T., ASAZUMA, T., YAMADA, H., MAEDA, H., and FUJIKAWA, K. (2001b): 'Glycosaminoglycan accumulation in primary culture of rabbit intervertebral disc cells', *Spine*, **26**, pp. 2653–2660

SATO, M., ASAZUMA, T., ISHIHARA, M., KIKUCHI, T., MASUOKA, K., ICHIMURA, S., KIKUCHI, M., KURITA, A., and FUJIKAWA, K. (2003): 'An atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS-scaffold) for the culture of annulus fibrosus cells from an intervertebral disc', *J. Biomed. Mater. Res.*, **64A**, pp. 248–256

SUMIDA, K., SATO, K., AOKI, M., MATSUYAMA, Y., and IWATA, H. (1999): 'Serial changes in the rate of proteoglycan synthesis after chemonucleolysis of rabbit intervertebral discs', *Spine*, **24**, pp. 1066–1070

Author's biography

MASATO SATO received his MD in 1991, and his PhD in Orthopaedic Surgery and Medical Engineering, in 2001 from National Defense Medical College, Japan. He has been chief of the department of Orthopaedic Surgery, Self Defense Force Yokosuka Hospital and also lecturer of the department of Medical Engineering, National Defense Medical College. His clinical specialties include Spine Surgery and Rheumatology. His main research activities include regenerative medicine for intervertebral disc, articular cartilage, and bone, using tissue engineering method. He is also interested in non-invasive measurement of biomechanical feature of cartilageous tissue.