

# JMAJ

## Japan Medical Association Journal

### CONTENTS

#### *JMA Policies*

- Policy Address  
Haruo UEMATSU ..... 305

#### *Regenerative Medicine*

- Regenerative Medicine for Cartilage Defects  
Mitsuo OCHI ..... 307
- The Use of Skin Regeneration Technique in the Treatment of  
Full-Thickness Skin Defects  
Norio KUMAGAI ..... 311
- Regenerative Medicine for the Cornea  
Shigeru KINOSHITA and Takahiro NAKAMURA ..... 317
- Regenerative Medicine for Sclerotic Disorders  
Toshikazu NAKAMURA ..... 322
- Regenerative Medicine for Cardiomyocytes  
Keiichi FUKUDA ..... 328
- Regenerative Medicine for Respiratory Diseases  
Tatsuo NAKAMURA ..... 333

#### *Centenarians*

- Implications of Research Findings Obtained from Centenarians  
Hiroshi SHIBATA ..... 338

#### *Abdominal Aortic Aneurysm*

- Forefront of Treatment for Abdominal Aortic Aneurysm  
Hiroshi YASUHARA ..... 344

# Policy Address

JMAJ 47(7): 305–306, 2004

Haruo UEMATSU

*President, Japan Medical Association*

*The 110th General Assembly of the JMA House of Delegates was held for two days from April 1 to 2 at the JMA Hall. Elections for the offices of the executive board were held on the first day and Dr. Haruo Uematsu (former president of the Osaka Medical Association) was elected as the 16th president of the JMA for the first time. The following is a main part of the policy address of Dr. Uematsu presented on April 2, 2004.*

Our country is facing a period of great changes as can be seen in the dispatch of Self-Defense Forces to Iraq by the government of Japan amidst an ongoing debate about revising the Japanese Constitution. Thus, health care reforms which are carried out during this period must reflect answers to the question, “What kind of country should Japan strive to become in the future?” Reforms that address only the immediate issues will not contribute to national happiness and prosperity.

## **Striving for Transparency in JMA Affairs and Earning the Trust of JMA Members**

We have been entrusted with the management of the JMA in times such as these and we are fully aware of the importance and the significance of this responsibility. The board members that you recently elected into office are capable, flexible, and dynamic individuals who have the confidence and recommendation of each local medical association. However, it is regrettable that no women members were elected as executive members. Reflecting on this turn of events, we would like each local medical association to actively recruit excep-

tional women, to foster their capabilities, and to send them to the JMA in future.

Since this is such an important time for the JMA, I believe that making the organization’s management very transparent while clarifying its decision-making process will raise the trust of our members. Executive members should address health insurance, long-term care insurance, community health care, and other issues through teamwork, and we are presently preparing to do this. Issues that may be overlooked by one or two individuals can be addressed without fail by a team of three or four members.

## **Firm Support for Social Security and a Universal Health Insurance Program**

There are many highly qualified, outstanding individuals with accumulated know-how within the Secretariat. I would like to pursue a policy whereby these talented individuals are utilized more often than has been in the past and to allow them to voice their opinions in policy decisions as needed. I believe that such a policy will not only be an added help for the executive members, but it will also serve as a means of encouragement for this important pool of out-

standing individuals.

But since the JMA is dedicated to improving public health care, I would like JMA's mainstay policy to be the firm support of social security and the national universal health insurance program. The foremost important element is to provide safe and high quality health care services efficiently. To accomplish this, some of the existing issues that come to mind are promoting the CME program for our members, enhancing medical ethics, and pursuing self-reviews.

Policies regarding these issues have been adequately debated and compiled into a report by the previous executive board, and what remains now is their actual implementation. In implementing these policies, we should observe the response of not only JMA members, but the opinions and response of the general public, while maintaining a very stringent stance when discussing these policies with members.

### **Raising Incentives to Join the JMA and Improving the Membership Ratio**

The most problematic area in health care services is pediatric care. This is especially true in the area of pediatric emergency care. Although pediatric emergency care is available throughout the country, the adequacy of these services varies according to geographical area. I hope that a reliable system of pediatric emergency care is established uniformly throughout the country. To achieve this, the problem of the maldistribution of physicians must be addressed.

Additionally, issues related to women physicians and employed physicians must be addressed as well.

Improving the membership ratio is an extremely important issue for the future of the JMA. Some of the notable problems that must be addressed are how to raise the understanding of younger and employed physicians about the JMA. Although the merits about joining are discussed, information that cultivates understanding about the kind of work the JMA is involved in and how it contributes to the world at large as well as what the incentives are for physicians to join the association must be disseminated. This information should not simply be a discussion about incentives, but measures must be taken to earn the trust of employed physicians.

### **Implementing Responsible Policies and Strategies by the Executive Board**

Among the issues that must be addressed, there is the issue regarding the JMARI (JMA Research Institute). I am fully aware of the fact that the JMARI is an effective and competent institute. However, I feel that it is the responsibility of the executive board to decide and implement JMA's policies; and the role of the JMARI is to carry out specific tasks, analyze data, and carry out surveys and studies that support the decisions made by the executive board. I think that it is irresponsible to leave the decision-making process in the hands of the institute.

# Regenerative Medicine for Cartilage Defects

JMAJ 47(7): 307–310, 2004

Mitsuo OCHI

*Professor, Department of Orthopaedic Surgery,  
Hiroshima University Graduate School of Biomedical Sciences*

**Abstract:** The treatment of full-thickness defects of articular cartilage remains a problem for orthopedic surgeons. It has been generally accepted that once articular cartilage is injured and forms a defect, the defect cannot be repaired and bordering intact cartilage undergoes degeneration which destroys facing intact cartilage and results in osteoarthritis. Several attempts to repair articular defects with hyaline cartilage have failed. Since the clinical reports of Brittberg *et al.* in 1994, autologous chondrocyte transplantation has raised the hopes and expectations of orthopedic surgeons that a breakthrough in the repair of damaged articular cartilage is imminent. In Brittberg *et al.*'s technique, cartilage slices were obtained by arthroscopy from an unloaded area of the femoral condyle; the associated chondrocytes increased in number in a monolayer culture after enzymatic digestion, and the chondrocytes in suspension were then injected into a cartilaginous defect and covered with a flap of the periosteum. According to their report, clinical results were satisfactory and a biopsy of the graft sites showed hyaline-like cartilage repair. However, we had several reservations about their technique in regard to the culture and transplantation procedure in terms of 1) de-differentiation of chondrocytes during a long cultivation, 2) uneven distribution of the grafted chondrocytes throughout the osteochondral defects, and 3) a high risk of leakage of grafted chondrocytes from the defects. We developed a new technique that enables the shift from cell transplantation to tissue transplantation. This technique creates a new cartilage-like tissue by the tissue-engineering technique in which autologous chondrocytes were embedded in atelocollagen gel and cultivated for about 3 weeks. We carefully selected atelocollagen gel as a three-dimensional culture material from the viewpoint of safety and non-immunogenicity, since atelocollagen gel had been used clinically for the treatment of skin wrinkles in plastic surgery and dermatology. Cultivation results in the proliferation of chondrocytes and the synthesis of an extracellular matrix consisting of chondroitin sulfate and type II collagen at transplantation. By 3 weeks of cultivation, the atelocollagen gel, including chondrocytes had acquired a jelly-like hardness. We have been using our technique since gaining the approval of the ethics committee in 1996. After 3 to 4 weeks' culture of autologous chondrocytes embedded in atelocollagen gel, a tissue-engineered cartilage was transplanted into a cartilage defect and covered with a periosteal flap, which was sutured with the deep cambium layer facing the subchondral bone plate. We followed up full-thickness cartilage defects of 36 knees from 34 patients treated with our procedure over a minimum period of 2 years. Clinical, arthroscopic and biomechanical results were relatively satisfactory. We conclude that transplanting tissue-engineered cartilage made by the tissue-engineering technique can promote restoration of the cartilage of the knee.

**Key words:** Cartilage injury; Cartilage repair; Three-dimensional culture; Regenerative medicine

---

This article is a revised English version of a paper originally published in the Journal of the Japan Medical Association (Vol. 129, No. 3, 2003, pages 336–338).

## Introduction

With the progress in tissue engineering and molecular biology, basic research in regenerative medicine aimed at regeneration of defective tissues or organs using autologous cells or tissues and their clinical application have become widespread in recent years. For the regeneration of tissues using tissue engineering techniques, it is generally recognized that cell transplants, biocompatible materials to serve as a foothold for growth of transplanted cells (i.e., scaffold), and growth factors are usually required, and studies on regeneration of a wide variety of tissues have been extensively undertaken in the field of medical care. Regenerative medical care has thus been frequently introduced by mass media such as newspapers, periodicals, and television and widely recognized among the public at large. Knowledge of regenerative medicine is now essential to the practice of medicine.

Repair of articular cartilage, which has little or no self-repairing capacity, has been taken up as a matter to be pursued in the field of orthopedic surgery. Articular cartilage comprises chondrocytes and surrounding extracellular matrix (such as type II collagen and proteoglycans) and is devoid of blood vessels and nerve tissues. The chondrocytes are poorly proliferative and produce little extracellular matrix. Once articular cartilage is damaged, the usual tissue repair process does not proceed with a consequent lack of restoration of hyaline cartilage.

When an articular cartilage defect occurs, it gives rise over time to degeneration of the surrounding and facing intact joint cartilage, which progresses in due course to become osteoarthritis. Consequently, pain and limitation of excursion occur and functional impairment of the knee joint ensues. This constitutes a significant clinical problem. Articular cartilage injury may be encountered not only in the practice of orthopedic surgery but in other clinical settings as well in the form of osteo-

chondritis dissecans in young subjects, traumatic cartilage injury, and osteoarthritis or rheumatoid arthritis in the elderly.

Currently, there is no other choice than artificial joint replacement for reconstruction of markedly destroyed articular function due to advanced osteoarthritis or rheumatoid arthritis. For the treatment of localized articular cartilage defects, a variety of procedures has been undertaken, including the drilling method and the microfracture technique aimed at inducing marrow cells from a subchondral bone, soft tissue grafting such as periosteum-perichondrium or meniscus transplantation, and osteochondral transplantation. None of these surgical procedures has proven to provide satisfactory reconstruction of a natural, smooth cartilage with hyaline cartilage at the site of defect.

In 1994, Brittberg *et al.* reported transplantation of cultured autologous articular chondrocytes utilizing monolayer cell cultures.<sup>1)</sup> Cartilage slices obtained by arthroscopy from an unloaded region of the knee joint were trypsinized to isolate chondrocytes, which were then incubated to grow into monolayer cultures. The grown chondrocytes were dispersed in suspension and injected into a cartilaginous defect covered with a flap of the periosteum. The technique is currently in widespread use on a commercial basis mainly in Europe and the United States.

According to their report, a postoperative biopsy of the graft sites showed hyaline-like cartilage repair. The technique thus marked a major breakthrough in the repair of damaged cartilage. Several problems, however, seem to be inherent in this technique, viz. whether chondrocytes which have de-differentiated during the cultivation *in vitro* may recover their function as chondrocytes post-transplantation, whether leakage of the grafted chondrocytes in suspension may occur from the defect site, and whether the grafted chondrocytes may become evenly distributed throughout the osteochondral defect.

To resolve these problems, we have devised a

technique for tissue-engineered cartilage-like tissue grafting, namely, transplantation of atelocollagen gel-embedded cultured autologous articular chondrocytes. Based on laboratory studies, its clinical application was initiated for the first time in the world in 1996.<sup>2-7)</sup> Gratifying postoperative results have been obtained, and clinical trials are scheduled to be conducted at several university hospitals.

### Transplantation of Atelocollagen Gel-Embedded Cultured Autologous Articular Chondrocytes

The atelocollagen gel is type I collagen purified from the bovine corium and causes little or no immune responses to it because the atelocollagen is deprived of the antigenic terminal telopeptide. The gel is a remarkably safe collagen that is already in clinical use in cosmetic surgery for such purposes as eliminating skin wrinkles.

The specifics of the procedure are as follows. Cartilage specimens are obtained by arthroscopy from an intra-articular detached cartilage fragment or an unloaded region of knee joint cartilage of the patient. The specimens are sliced and trypsinized/collagenase-digested to isolate chondrocytes. The collected cells are then suspended in an atelocollagen gel and gelled, followed by incubation with a culture medium containing the patient's serum and antibiotics for 3 weeks. The embedded chondrocytes grow in the atelocollagen gel and liberate extracellular matrix to have the gel acquire a jelly-like hardness and turn into a cartilage-like tissue.

Three weeks after harvest of the autologous cartilage, the chondral defect lesion is exposed via arthrotomy, the chondrocyte-atelocollagen gel prepared *in vitro* is transplanted in the defect, and the lesion is covered by a sutured periosteal flap taken from the proximal medial tibia. One to two weeks after the transplantation, passive movement of the joint is begun. Partial weight-bearing is introduced at 4 weeks

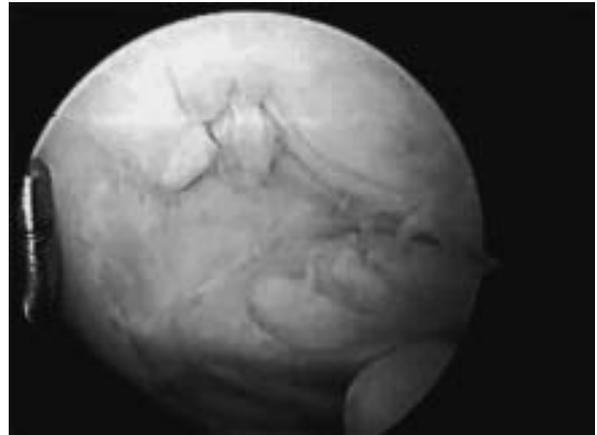


Fig. 1 Osteochondritis dissecans of the femoral medial condyle of the right knee in a 27-year-old man  
Note the cartilage defect extends as deep as the surface of subchondral bone.

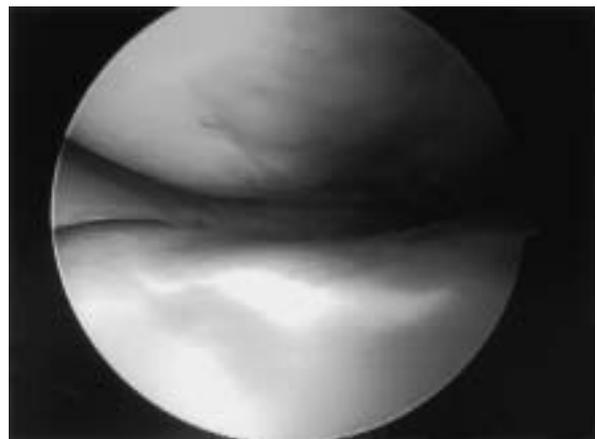


Fig. 2 Two years after transplantation of atelocollagen gel-embedded cultured autologous articular chondrocytes  
The defect is repaired with cartilage-like tissue with a smooth surface.

post-operation and is gradually increased to full weight-bearing with muscle training at 6–8 weeks post-operation.

In a laboratory study with human chondrocytes collected at an operation of artificial joint replacement, we confirmed and reported that human chondrocytes cultured in atelocollagen gel retained their three-dimensional structure and grew while maintaining their usual round shapes without incurring de-differentiation and

produced/released extracellular matrix such as type II collagen and chondroitin sulfate.

Inasmuch as chondrocytes are transplanted as embedded/cultured in the solid gel medium, there is practically no cell leakage from the periosteal sutured margin, unlike transplantation of cells in suspension. Leakage of chondrocyte transplant is thus quite unlikely even on articular movements, unless gel leakage results from significant periosteal damage; this constitutes a great advantage. The procedure is also advantageous in that it provides apparently greater uniformity of chondrocyte transplant as compared with the chondrocyte transplantation in suspension.

Treatment with this procedure has been performed on a total of 70 knees, with relatively satisfactory postoperative outcomes (Figs. 1 and 2). The patients were followed by periodic arthroscopic observations postoperatively, and the graft sites were confirmed to gradually increase in hardness to become as firm as the surrounding normal cartilage. On MRIs, the graft sites were shown over time to gain brightness close to that of surrounding normal cartilage.

The method's drawbacks are: the limited quantity of chondrocytes obtainable, the long period required for the transplanted cartilage to mature, and the two-stage operation to complete the procedure. Further spread of cultured autologous chondrocytes tissue grafting can be expected in the future upon improvement of the cultivation technique, development of a better transplant carrier supplanting the atelocollagen, and a technique enabling autologous stem cell differentiation into chondrocytes. Cooperation and joint research with other fields such as medicoengineering and molecular biology are essential for the development of the method.

## Conclusion

With the increases in the elderly population and the sports population in recent years,

opportunities to treat patients suffering from articular cartilage injury have increased. While it is not easy to repair an articular cartilage injury with the original hyaline cartilage, we devised a tissue-engineered technique for transplantation of atelocollagen gel-embedded cultured autologous articular chondrocytes and have been applying it in clinical settings. Though the postoperative follow-ups have not been long enough, satisfactory results have been obtained with this technique. Exploration of the current problems involved and various approaches to attain more satisfactory outcomes for cartilage repair are under way.

## REFERENCES

- 1) Brittberg, M., Lindahl, A., Nilsson, A. *et al.*: Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994; 331: 889–895.
- 2) Ochi, M., Uchio, Y., Kawasaki, K. *et al.*: Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. *J Bone Joint Surg Br* 2002; 84: 571–578.
- 3) Ochi, M., Uchio, Y., Tobita, M. *et al.*: Current concepts in tissue engineering technique for repair of cartilage defect. *Artif Organs* 2001; 25: 172–179.
- 4) Matsusaki, M., Ochi, M., Uchio, Y. *et al.*: Effects of basic fibroblast growth factor on proliferation and phenotype expression of chondrocytes embedded in collagen gel. *Gen Pharmacol* 1998; 31: 759–764.
- 5) Kawasaki, K., Ochi, M., Uchio, Y. *et al.*: Hyaluronic acid enhances proliferation and chondroitin sulfate synthesis in cultured chondrocytes embedded in collagen gels. *J Cell Physiol* 1999; 179: 142–148.
- 6) Katsube, K., Ochi, M., Uchio, Y. *et al.*: Repair of articular cartilage defects with cultured chondrocytes in Atelocollagen gel. Comparison with cultured chondrocytes in suspension. *Arth Orthop Trauma Surg* 2000; 120: 121–127.
- 7) Uchio, Y., Ochi, M., Matsusaki, M. *et al.*: Human chondrocyte proliferation and matrix synthesis cultured in Atelocollagen gel. *J Biomed Mater Res* 2000; 50: 138–143.

# The Use of Skin Regeneration Technique in the Treatment of Full-Thickness Skin Defects

JMAJ 47(7): 311–316, 2004

Norio KUMAGAI

*Professor and Chairman, Department of Plastic and Reconstructive Surgery,  
St. Marianna University School of Medicine*

**Abstract:** A total of 434 patients have been treated with cultured epithelium transplantation between March 1985 and April 2003, 285 patients by autografting and 149 patients by allografting. Nevertheless, this approach of cultured epithelium is confined only to the epidermis and not the dermis. This results in poor “graft take” and failure of skin regeneration even by autografting in full-thickness skin defects. Regeneration of the dermis is essential for improving the “take” rate of cultured epithelia. Currently, transplantation of cultured autologous epithelium over a previously transplanted dermal allograft is an effective method of treatment for full-thickness skin defects such as third-degree burns and giant nevus resection wounds. However, there are problems with this technique, such as rejection of allograft skin and a short supply of allograft skin. Attention has shifted to the use of cell-free dermis, the development of cultured dermis and cultured skin by tissue engineering techniques. This article describes the present status of skin regeneration techniques for the treatment of full-thickness skin defect via cultured autologous epithelium transplantation and the experience of this department in the treatment of full-thickness skin defects.

**Key words:** Skin regeneration technique; Cell culture; Skin; Tissue engineering

## Introduction

Skin grafting is a widely used operative procedure in both plastic surgery and general surgery, but it has limitations in the treatment of extensive burn wounds in which the skin left

for harvesting is inadequate.

In recent years, many patients have benefited from skin regeneration techniques since the introduction of “cultured epidermal transplantation” for wound coverage in extensive full-thickness skin defects. However, the thera-

---

This article is a revised English version of a paper originally published in the Journal of the Japan Medical Association (Vol. 129, No. 3, 2003, pages 339–342).

Table 1 Breakdown of Cases Treated with Cultured Autologous or Allogeneic Epidermal Transplantation (April 1985 to April 2001)

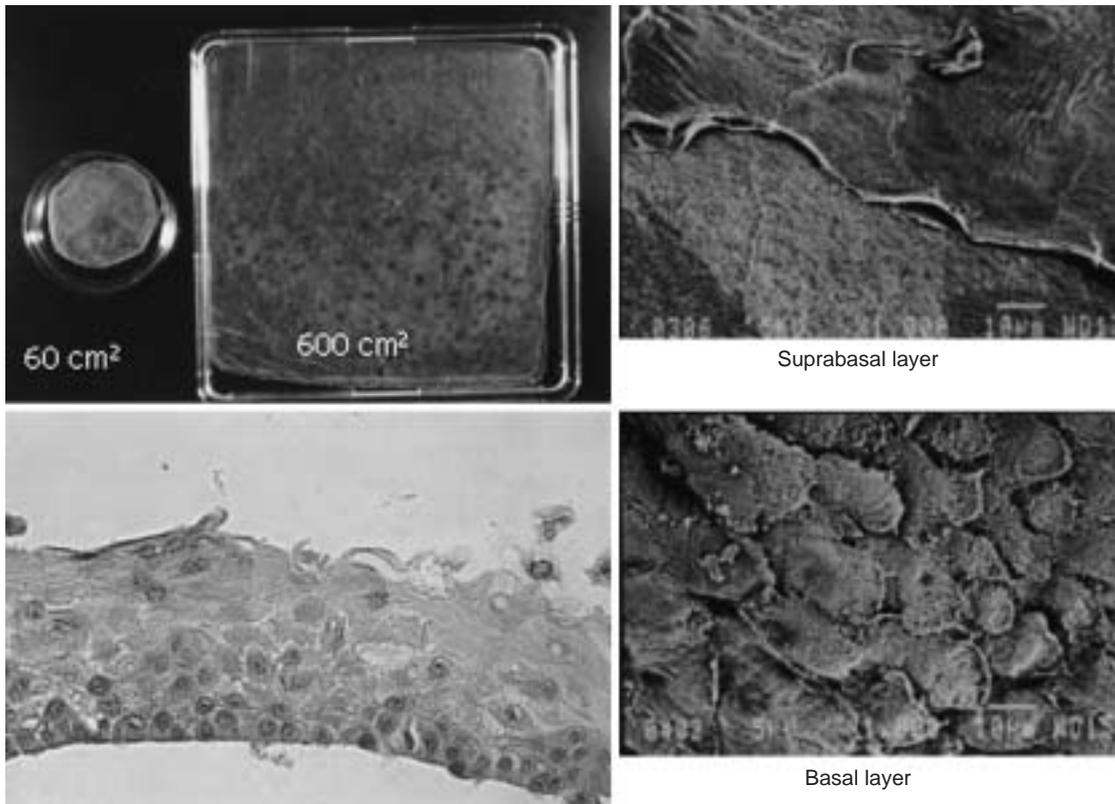
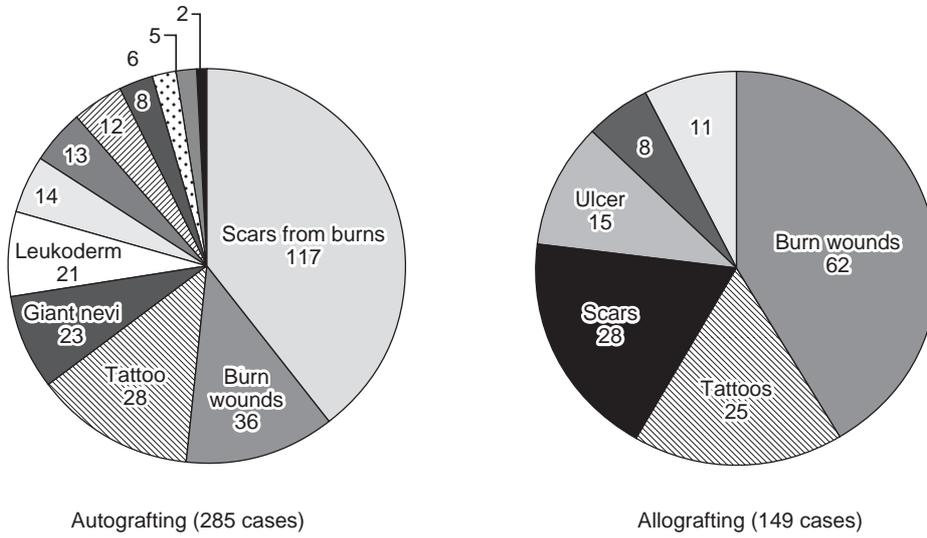


Fig. 1 Cultured epithelium and scanning electron micrographs

peutic approach for the above technique is confined to the epidermis alone without the dermal components which has its own problems. This article describes the achievements and the present status of skin regeneration techniques in the treatment of full-thickness skin defects.

### Results of Clinical Application of Cultured Epithelium

The author and colleagues started clinically applying autologous cell cultured transplantation in April 1985. This technique has been used for treating various skin disorders such as burns, scars, nevi, vitiligo and tattoos. Of the total of 434 patients that were treated, 285 patients underwent autografting and 149 patients received allografting (Table 1). Our laboratory's accomplishments have now made it possible to yield enough cultured epidermis to cover the whole body surface area ( $1.6\text{m}^2$ ) in one month of incubation from a skin graft of about  $10\text{cm}^2$  (Fig. 1). Such mass epidermal cultivation from a small quantity of skin graft can be obtained because the skin contains actively proliferative stem cells with high differentiating potential. It has been demonstrated that follicular multipotent stem cells capable of differentiating into internal and external root sheaths, follicle, sebaceous gland and epidermis which exist not only in the stratum basal epidermis, but also in the follicular bulge.<sup>1)</sup>

Clinical application of cultured epithelia began in 1980. In 1984, two children aged 5 and 6 years old who sustained third-degree burns over 97% of their total body surface area were saved by autologous cultured human epithelium transplantation.<sup>2)</sup>

### Discussion

Rheinwald and Green's<sup>3)</sup> established a mass culture method of epidermal cells by using irradiated 3T3 cells as the feeder in 1975, and the subsequent groundbreaking success of its clinical application may be regarded as the

very beginning of current skin regeneration techniques.

### Effects of Dermal Substance in Skin Regeneration

Interaction between the epidermis and the dermis play an important role in skin regeneration when autologous cultured epithelia are transplanted onto split-thickness skin wounds retaining the dermis, nearly 100% of the thin transplants take and regenerate to become normal skin within one week. The laminin 5, type IV collagen and type VII collagen which are components of basal layer between wound bed and cultured epithelia are stained. Furthermore, rete ridges are formed. In cases where autologous cultured epithelia are transplanted onto full-thickness skin defect wounds devoid of dermal components, the graft take is extremely low. At 20% one week after transplantation, the graft shows no appreciable change in thickness or formation of the basal layer. The transplant gains in thickness and the basal layer finally forms several weeks later. There are no formation rete ridges, the border of the wound bed is even, and the skin shows characteristics of cicatricial epithelium, such as hyperkeratosis, hypokeratosis, and dermatoglyphic hypoplasia. As such it takes five months for the basement membrane and continuous fibers to resume their normal morphologic features; the region is liable to develop vesicles and to be readily detached during this period. The cultured epithelial cells do not take on the adipose tissue layer or granulation tissue wounds.

### Reconstruction of the Dermis

To raise the graft take rate for cultured epithelia to enable skin regeneration, it is important to generate wound bed formation to serve as a scaffold for cultured epidermal transplants and to enable regeneration of the dermal components. There have been two different methods reported for the generation of the dermis:

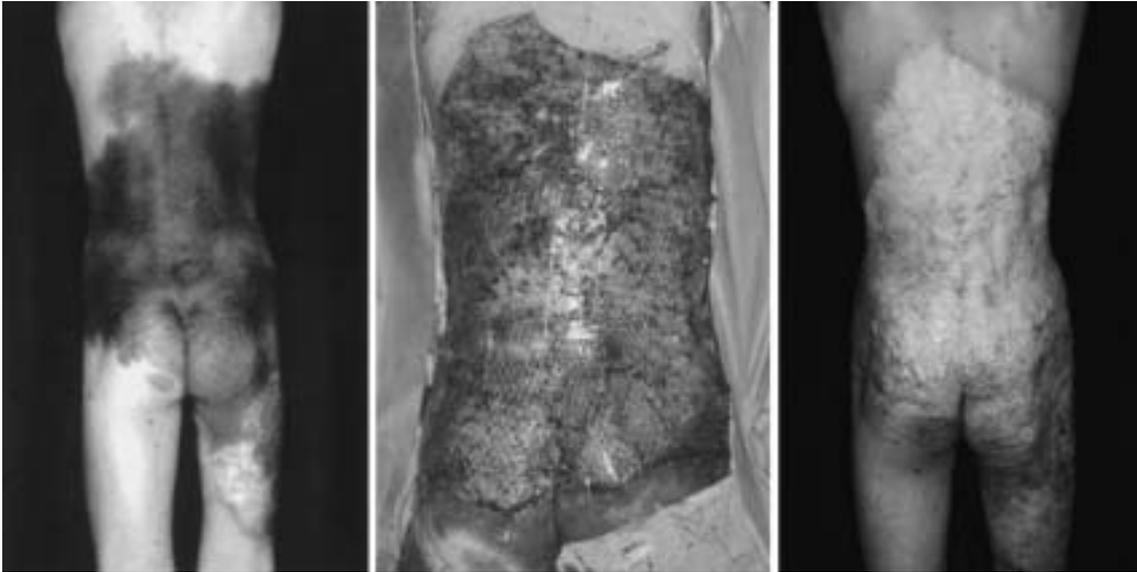


Fig. 2 Treatment of giant nevi by transplantation of skin allografts and cultured autologous epithelial grafts (Left: preoperative; center: cultured autologous epithelia transplanted over skin allografts; right: 7 years after treatment)

one is to prepare dermal components over the wound bed in advance, followed by cultured autologous epithelia grafting and the other is to prepare a cultured integrated epidermis-dermis skin transplant *in vitro* by the so-called tissue engineering technique.

### 1. Utilization of skin allografts

Cuono *et al.*,<sup>4)</sup> treated third-degree burns by covering the wound surface with allogeneic skin grafts after resecting the necrotized wound, followed by coverage with cultured autologous epithelium over the dermal allograft. They were successful in preventing burn wound infection and achieved graft take rates of 80% to more than 85% owing to the transplantation over the dermal components. With the same procedure, the present author and colleagues succeeded in saving two children from extensive (85% and 90% body surface area) burn wounds.<sup>5)</sup>

In the treatment of giant congenital nevi, the lesions are resected in full thickness of the skin extending to the underlying adipose tissue layer. Transplantation of cultured autologous epithelium onto such wounds results in poor graft take rates. Therefore, the lesions are

treated according to the procedure for third-degree burns. In view of intense allograft rejection of the host with nevi, unlike immunotolerant patients with burn wounds, allografts were cryopreserved for more than one month prior to transplantation in order to suppress their antigenicity. Cultured autologous epithelium was transplanted onto the dermal allograft about ten days after transplantation of the latter (Fig. 2). As a result, the graft take rate for cultured autologous epidermis improved.<sup>6)</sup> Therefore, the use of dermal allografts is the most practical because they improve take rates when cultured autologous epithelium is transplanted over dermal allografts and the treated skin presents histological features similar to those of the dermis.

In recent years, cell-free dermal preparations from epidermis-dermis deprived of cellular components have become commercially available in various other countries and are used as intra-tissue filling materials mainly in plastic surgery. These materials are immunologically inert and have been reported to promote the take of cultured epithelium.<sup>7)</sup> Treatment with cultured autologous epithelium used

in combination with skin allografts or cell-free dermal preparations seems to be efficacious as a therapeutic technique for full-thickness skin defects. However, these materials are not readily available in this country and there may be a hidden risk of viral infections of human origin.

## 2. Dermal reconstruction by tissue engineering technique

In attempting skin reconstruction utilizing tissue engineering, the type of matrix that should be used as a scaffold for growing epidermal cells and skin fibroblasts must be determined. Fibrin glue is a preparation employed in daily clinical practice, and epidermal cells cultured on this glue retain greater capacity of maintaining epidermal stem cells, compared to the 3T3 feeder layer method. The epidermal cells so cultured are easier to handle and their basement membrane structure can be preserved. It has been documented by Ronfard, *et al.*<sup>8)</sup> that when transplanted over a dermal allograft, epidermal cells cultured on fibrin glue were closer in appearance and histological features to normal skin than when transplanted alone. Ongoing clinical application of cultured cells containing epidermal keratinocytes and fibroblasts mediated by fibrin glue at this department has shown improved graft take rates. Some matrix constituents as well as fibroblasts may be contributing to the take rate improvement, but the costliness of fibrin glue poses a practical problem.

Meanwhile, studies have been undertaken to reconstruct the skin *in vitro* by culturing epidermal keratinocytes and fibroblasts with spongy artificial dermis mainly consisting of atelocollagen as scaffold.<sup>9,10)</sup> Clinical application of cultured dermis containing cultured allogeneic fibroblasts on spongy sheets comprising hyaluronic acid and atelocollagen developed by Kuroyanagi at Kitasato University is in progress<sup>11)</sup> as part of a skin regeneration study under the governmental millennium project in Japan in 2000.

Ulcer surfaces covered with cultured dermis transplant show accelerated healing as a result of promoted vascularization due to vascular endothelial growth factor (VEGF) secreted by the fibroblasts. However, wound bed formation to improve the graft take rate of cultured epithelium has not been achieved as yet. Normal dermis contains networks of numerous microvasculatures and has a complicated cytokine network. Not only fibrous components such as fibroblasts and collagen fibers, but also the vascular system and various cytokines are involved in the regeneration of dermis, so that it is difficult at present to prepare cultured dermis that can substitute for the cutis vera *in vivo*. The introduction of multipotent tissue stem cells and supplementation with humoral factors will be needed in the future.

## Conclusion

The use of skin regeneration for the treatment of full-thickness skin defect is still under development and investigation. As mentioned above, remarkable results have been achieved in the clinical setting. Against this background, the environment surrounding regeneration skin technique has been developing rapidly, not only in academic but also in medical industries as well. Widespread clinical application of cultured skin prepared with tissue engineering techniques will be realized in the near future. Furthermore, value-added cultured skin preparations such as those enabling treatment of diseases with gene-transferred epidermal stem cell grafting may become clinically available.

## REFERENCES

- 1) Oshima, H., Rochat, A., Kedzia, C. *et al.*: Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* 2001; 104: 233–245.
- 2) Gallico, G.C., O'Connor, N.E., Compton, C.C. *et al.*: Permanent coverage of large burn wounds with autologous cultured human epithelium. *N Engl J Med* 1984; 311: 448–451.

- 3) Rheinwald, J.G. and Green, H.: Serial cultivation of strains of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cells* 1975; 6: 331–343.
- 4) Cuono, C.B., Langdon, R., Birchall, N. *et al.*: Composite autologous-allogeneic skin replacement: Development and clinical application. *Plast Reconstr Surg* 1987; 80: 626–637.
- 5) Matsuzaki, K., Kumagai, N., Fukushi, N. *et al.*: Treatment of extensive burn wound patients with skin allograft/cultured autologous epithelium; assessments of the grafted sites 4 years after the treatment. *Keisei-geka (J Plast Surg)* 1995; 38: 193–200. (in Japanese)
- 6) Kumagai, N., Oshima, H., Tanabe, M. *et al.*: Treatment of giant congenital nevi with cryopreserved allogeneic skin and fresh autologous cultured epithelium. *Ann Plast Surg* 1997; 39: 483–488.
- 7) Rennekapff, H.O., Kiessig, V., Griffey, S. *et al.*: Acellular human dermis promotes cultured keratinocyte engraftment. *J Burn Care Rehabil* 1997; 18: 535–544.
- 8) Ronfard, V., Rives, J.M., Neveux, Y. *et al.*: Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix. *Transplantation* 2000; 70: 1588–1598.
- 9) Hansbrough, J.F., Boyce, S.T., Cooper, M.L. *et al.*: Burn wound closure with cultured autologous keratinocytes and fibroblasts attached to a collagen-glycosaminoglycan substrate. *JAMA* 1989; 262: 2125–2130.
- 10) Kuroyanagi, Y., Kenmochi, M., Ishihara, S. *et al.*: A cultured skin substitute composed of fibroblasts and keratinocytes with collagen matrix: preliminary results of clinical trials. *Ann Plast Surg* 1993; 31: 340–349.
- 11) Kuroyanagi, Y.: Reconstruction of skin by regenerative tissue engineering; Present status of the development of cultured skin substitutes. *Seitaizairyo (Biomaterials)* 2001; 19: 166–176. (in Japanese)

# Regenerative Medicine for the Cornea

JMAJ 47(7): 317–321, 2004

Shigeru KINOSHITA\* and Takahiro NAKAMURA\*\*

\**Professor, Department of Ophthalmology, Kyoto Prefectural University of Medicine*

\*\**Department of Ophthalmology, Kyoto Prefectural University of Medicine*

**Abstract:** Regenerative medicine is a new field of medical science that aims to regenerate tissues or organ functions by utilizing such means as stem cells. The cornea is an avascular, transparent structure consisting primarily of three layers, the epithelium, stroma, and endothelium, making it a markedly distinctive body tissue. For the cornea's regeneration, two approaches are considered feasible: regeneration of the cellular layers from existing tissue specific stem cells or regeneration by induction of ocular tissues from embryonic stem (ES) cells. This article summarizes current approaches to corneal epithelial regeneration, with particular reference to regenerative medicine as applied to ocular surface reconstruction techniques, such as cultured corneal epithelium transplantation using amniotic membrane as a substrate and transplantation of cultured oral mucosal epithelium.

**Key words:** Amniotic membrane; Corneal epithelium; Oral mucosal epithelium; Ocular surface reconstruction

## Introduction

Following the settlement of gene decoding studies symbolized by the Human Genome Project in the twentieth century, the leading topic has been shifting to post-genome research since the beginning of the twenty-first century. The field of regenerative medical care and regenerative medicine is suddenly in the spotlight. Mass media coverage of regenerative medicine in recent years has been striking. Therefore, regenerative medicine researchers are responsible for providing accurate information.

Regeneration is “a phenomenon in which damaged/lost tissues are repaired with special

cells capable of mitotic division (e.g., stem cells) and restored to their original states”. Ophthalmology study subjects may be roughly divided into anterior segment domain (corneal disorders, cataracts) and posterior segment domain (retinal degeneration, optic neuropathy). In this paper, we would like to review our findings and state our thoughts regarding the future of regenerative medicine for the cornea (epithelium), with which our study group is actively involved.

## Structure of the Cornea

In vertebrates, including humans, eyes func-

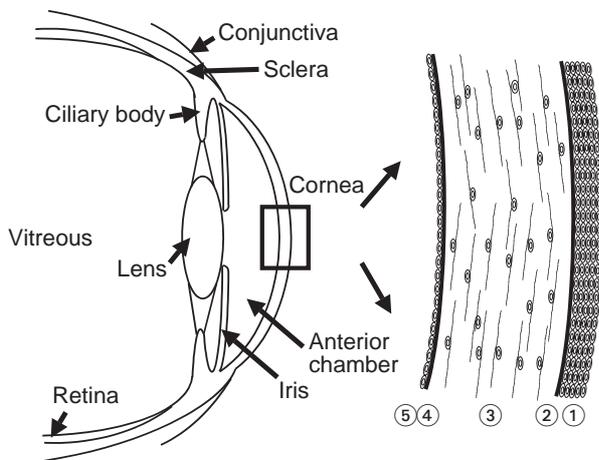


Fig. 1 Structures of the eyeball and cornea

- ① Corneal epithelium, ② Bowman's membrane,
- ③ Corneal stroma, ④ Descemet's membrane, and
- ⑤ Corneal endothelium

tion to take charge of the sense of sight, the sense that, it is said, provides humans with most of their information about the outside world. Eyes are important information-perceiving organs for conveying visual information to the brain. The cornea of the eye is highly transparent and serves as a barrier. Its principal physiological function consists in introducing light rays into the eyeball to permit focusing on an image on the retina. The cornea also serves as an outer physical and biological layer to protect the eyeball from extraneous foreign bodies and invasion by microbial pathogens.

The cornea may be divided into three cell layers from outer to inner: the epithelium, stroma, and endothelium. The outermost corneal epithelium is nonkeratinized, stratified and squamous, is of ectodermal origin, and comprises the ocular surface structure together with the peripheral limbal epithelium and conjunctival epithelium.<sup>1)</sup> The corneal stroma is an avascular, transparent tissue, accounting for more than 90% of the cornea's total thickness, and consists mainly of keratocytes and such extracellular matrix as collagen and proteoglycans. The corneal endothelium is a single layer of cells of neural crest origin, like the corneal stroma, and is situated at the inner-

most aspect of the cornea. It serves to regulate hydration of the cornea through an active pumping function and maintains corneal transparency (Fig. 1).

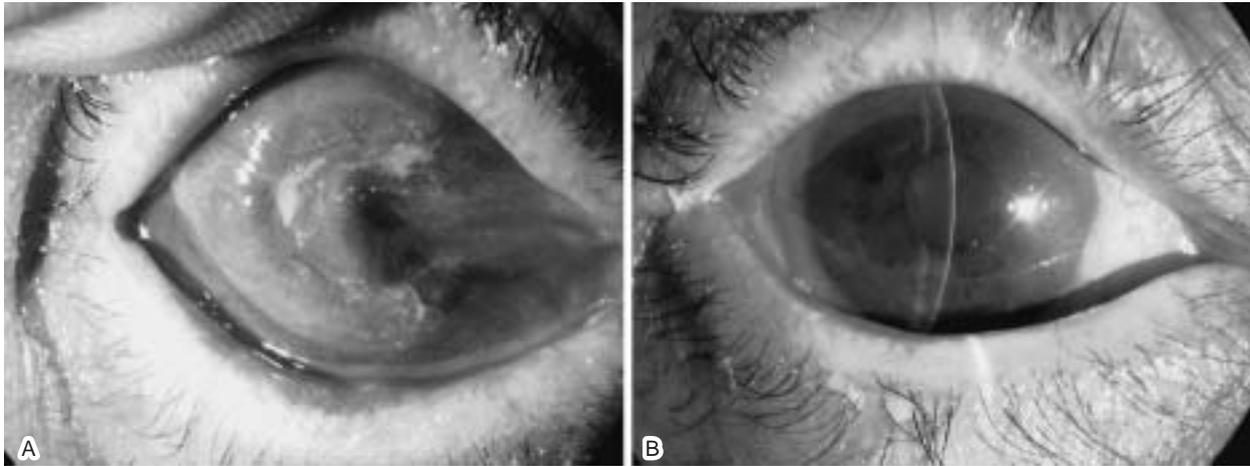
## Regeneration of the Corneal Epithelium

Severe ocular diseases refractory to various treatments among corneal disorders currently include Stevens-Johnson syndrome, ocular cicatricial pemphigoid, and chemical burns. It has been documented that corneal epithelial stem cells are localized in the radial plicate structure, called the palisade of Vogt, which is situated in the pericorneal region (corneal limbus).<sup>2,3)</sup> In severe ocular surface disorders, corneal epithelial stem cells are extensively damaged with a consequent failure in supply of normal corneal epithelial cells, eventually causing coverage of the ocular surface along with inflammation of the peripheral conjunctival epithelium and marked visual impairment.

Attempts to regenerate the corneal epithelium have been made by various groups of researchers as a therapeutic strategy against these disorders. A variety of methods for preparing transplant sheets has been investigated, e.g., a sheet prepared with corneal epithelial cells alone,<sup>4)</sup> a sheet comprising corneal epithelial cells cultured on a substrate such as a high polymer, collagen or fibrin,<sup>5-7)</sup> and a corneal epithelial cell sheet using biomaterial such as an amniotic membrane as a substrate.<sup>8-10)</sup> Our department has conducted studies aimed at preparing cultured epithelial sheets on the amniotic membrane.

### 1. From *in vitro* to *in vivo* – Cultured corneal epithelium transplantation

Our study team succeeded in preparing sheets of differentiated, stratified corneal epithelium transplantable onto the ocular surface by cultivating corneal epithelial stem cells from a rabbit on the amniotic membrane in an animal model.<sup>10)</sup> The corneal epithelial stem cells were



A: Before operation. There are persistent corneal epithelial defects covered with inflammatory, pathologic conjunctival tissue, resulting in a marked loss of vision.

B: After operation. Anterior segment 8 months after transplantation of cultured corneal epithelium. The ocular surface is covered with cultured corneal epithelial graft, with retained transparency and recovery of visual acuity.

Fig. 2 Cultured corneal epithelium transplantation in the treatment of ocular cicatricial pemphigoid

co-cultured with 3T3 fibroblasts that were pre-treated with mitomycin C to suppress proliferative activity in the process of preparation. The cultured corneal epithelial sheet on the amniotic membrane was autotransplanted into the same rabbit, was taken over the ocular surface, and proved to maintain its transparency.

Based on the results of the basic data obtained from laboratory animals, we started clinical application of the allografting of cultured corneal epithelium in patients with severe ocular surface disorders at this department in 1999 after scrupulously obtaining informed consent in accordance with the approval of the institutional ethics committee of the university. The surgical procedure was as follows. We removed the conjunctival tissue from the cornea and also removed the subconjunctival tissue to expose the corneal stroma. We then secured the cultured allocorneal epithelium on amniotic membrane onto the corneal surface with sutures placed on the limbus. The allografting was performed on 34 eyes with severe ocular surface disorders, including acute-phase cases contraindicated for conventional surgical intervention. Forty-eight hours after the transplan-

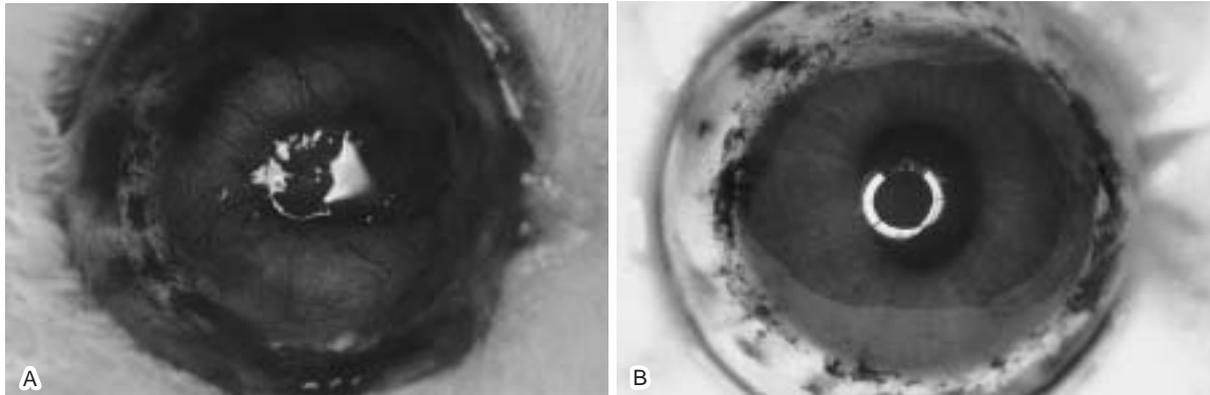
tation, graft survival was confirmed in all the patients who underwent cultured allocorneal epithelium transplantation (Fig. 2).<sup>11)</sup>

Corneal surfaces were cured and retained transparency in most cases, but because the transplants are allogeneic, problems such as postoperative rejection and infections have affected therapeutic outcomes. The fate of the transplanted cultured corneal epithelium remains uncertain, stressing the need for long-term follow-up of the cases.

## 2. From allo- to auto- — Cultured oral mucosal epithelium transplantation

For the treatment of severe ocular surface disorders, surgical reconstructive procedures have been developed, such as keratoepithelioplasty, limbal transplantation, and cultured corneal epithelium transplantation using allogeneic cells. Because severe ocular surface disorders are frequently bilateral, it is infeasible in such cases to attain reconstruction with autologous corneal epithelium, which eventually requires transplantation of allogeneic tissue grafts.

Therefore, taking note of oral mucosal epi-



A: Before operation. The ocular surface structure is covered with conjunctival tissue, presenting a feature of severe ocular surface disorder.

B: Anterior segment at 10 days after operation. The ocular surface is covered with a cultured oral mucosal epithelial sheet with retained transparency and stability.

Fig. 3 A rabbit cultured oral mucosal epithelial transplantation model

thelium among many other potential sources of cells in view of cytobiological characteristics and ease of tissue collection, we prepared cultured rabbit oral mucosal epithelial sheets on amniotic membrane and autotransplanted them onto rabbit ocular surfaces to assess their usefulness. Rabbit oral mucosal epithelium cultures on amniotic membrane demonstrated survival and growth of the epithelium. In addition, the cultured epithelium formed a stratified epithelium consisting of 5 to 6 layers of cells, resembling the normal corneal epithelium.

In rabbits that received transplantation of an autologous oral mucosal epithelial sheet onto the cornea, the reconstructed ocular surface was practically as transparent as the cultured corneal epithelial sheet. The autografted mucosal epithelial sheet retained its transparency and was confirmed to be surviving on the ocular surface 48 hours after the transplantation. On Day 10 post-transplantation, the autografted mucosal epithelium was demonstrated to have spread outward. It was thus confirmed that the oral mucosal epithelial sheet cultured *in vitro* was taken and spread on the ocular surface *in vivo* and its transparency restored (Fig. 3).

This therapeutic concept of ocular surface reconstruction with cultured oral mucosal epithelium sheet in the treatment of intractable,

bilateral ocular surface disorders, though still at the animal experiment level, represents a novel surgical procedure.<sup>12)</sup> This procedure is now in the stage of clinical application to humans in collaboration with the Department of Dentistry, and attempts at epithelial repair in acute phase corneal alkali burn have been successful in a clinical trial.<sup>13)</sup>

## Conclusion

The success in cultured cell transplantation from *in vitro* to *in vivo* has brought about a paradigm shift that operative treatment is now feasible even in acute-phase severe ocular surface disorders that had been contraindicated for surgical intervention. The idea of expanding the source of cells, furthermore, is leading to the possibility of a major breakthrough in surgical reconstruction of the ocular surface structure. This paper has presented our studies on regeneration of the corneal epithelium. As the structure of the cornea comprises three cellular layers, the epithelium, stroma, and endothelium, studies on regeneration of the corneal stroma and corneal endothelium are in progress at this department in tandem with corneal epithelial regeneration studies.

## REFERENCES

- 1) Thoft, R.A.: Conjunctival transplantation. *Arch Ophthalmol* 1977; 95(8): 1425–1427.
- 2) Schermer, A., Galvin, S. and Sun, T.T.: Differentiation-related expression of a major 64K corneal keratin *in vivo* and in culture suggests limbal location of corneal epithelial stem cells. *J Cell Biol* 1986; 103: 49–62.
- 3) Cotsarelis, G., Cheng, S.Z., Dong, G. *et al.*: Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell* 1989; 57(2): 201–209.
- 4) Gipson, I.K. and Grill, S.M.: A technique for obtaining sheets of intact rabbit corneal epithelium. *Invest Ophthalmol Vis Sci* 1982; 23: 269–273.
- 5) Ohji, M., Kinoshita, S., Watanabe, K. *et al.*: Corneal epithelium culturing on type IV collagen sheet. *Folia Ophthalmol Jpn* 1991; 42: 784–788. (in Japanese)
- 6) Friend, J., Kinoshita, S., Thoft, R.A. *et al.*: Corneal epithelial cell cultures on stromal carriers. *Invest Ophthalmol Vis Sci* 1982; 23: 41–49.
- 7) Rama, P., Bonini, S., Lambiase, A. *et al.*: Autologous fibrincultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency. *Transplantation* 2001; 72: 1478–1485.
- 8) Tsai, R.J., Li, L.M. and Chen, J.K.: Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med* 2000; 343: 86–93.
- 9) Shimazaki, J., Aiba, M., Goto, E. *et al.*: Transplantation of human limbal epithelium cultivated on amniotic membrane for the treatment of severe ocular surface disorders. *Ophthalmology* 2002; 109: 1285–1290.
- 10) Koizumi, N., Inatomi, T., Quantock, A.J. *et al.*: Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. *Cornea* 2000; 19(1): 65–71.
- 11) Koizumi, N., Inatomi, T., Suzuki, T. *et al.*: Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. *Ophthalmology* 2001; 108: 1569–1574.
- 12) Nakamura, T., Endo, K., Cooper, L.J. *et al.*: The successful culture and autologous transplantation of rabbit oral mucosal epithelial cells on amniotic membrane. *Invest Ophthalmol Vis Sci* 2003; 44(1): 106–116.
- 13) Nakamura, T., Inatomi, T., Sotozono, C., Amemiya, T., Kanamura, N. and Kinoshita, S.: Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. *Br J Ophthalmol* in press.

# Regenerative Medicine for Sclerotic Disorders

JMAJ 47(7): 322–327, 2004

Toshikazu NAKAMURA

*Professor, Division of Molecular Regenerative Medicine,  
Course of Advanced Medicine, Osaka University Graduate School of Medicine*

**Abstract:** Hepatocyte growth factor (HGF) was discovered in 1984 as a molecular entity of hepatic regeneration factor, which had long been sought after. In ensuing studies, HGF has been proven to enhance the growth and motility of hepatocytes and other functional cells and to induce morphogenesis represented by tubular structures. Furthermore, HGF is endowed with potent anti-apoptotic activity, thereby preventing various tissue injuries. HGF has been demonstrated in a wide variety of animal disease models to be remarkably effective in the prevention and treatment of acute and chronic organ disorders. It has marked curative/ameliorative effects in chronic diseases, especially such sclerotic disorders as liver cirrhosis, renal failure, nephrosclerosis, lung fibrosis and cardiomyopathy for which there is no definitive treatment to date. The remarkable effectiveness of HGF stems from its ability as a tissue-organizing molecule to provide functional improvement by inhibiting fibrosis and reconstructing normal tissues, as well as from its potent anti-apoptotic activity. These show that HGF is an intrinsic tissue repair factor.

**Key words:** Hepatocyte growth factor (HGF); Intrinsic regeneration factor; Lung fibrosis; Liver cirrhosis; Chronic renal failure

## Introduction

Ingenious tissue regeneration mechanisms in mammals are roughly divided into two distinct systems. One is a system in which undifferentiated, vigorously proliferative stem cells assume the principal role in tissue regeneration. It operates to regenerate and repair tissues comprising differentiated cells that are no longer capable of proliferation, such as the hemopoietic tissue

of bone marrow, nerve tissues and muscles. The other, termed the simple duplication system, is the regeneration system for tissues whose cellular components are mature and differentiated, yet vitally capable of proliferation as seen in the regeneration of parenchymal organs such as the liver, kidneys, and lungs. Therefore, for organs with complicated multicellular architecture such as the liver, kidneys, and lungs, treatment of an injury by activation

---

This article is a revised English version of a paper originally published in the Journal of the Japan Medical Association (Vol. 129, No. 3, 2003, pages 360–364).

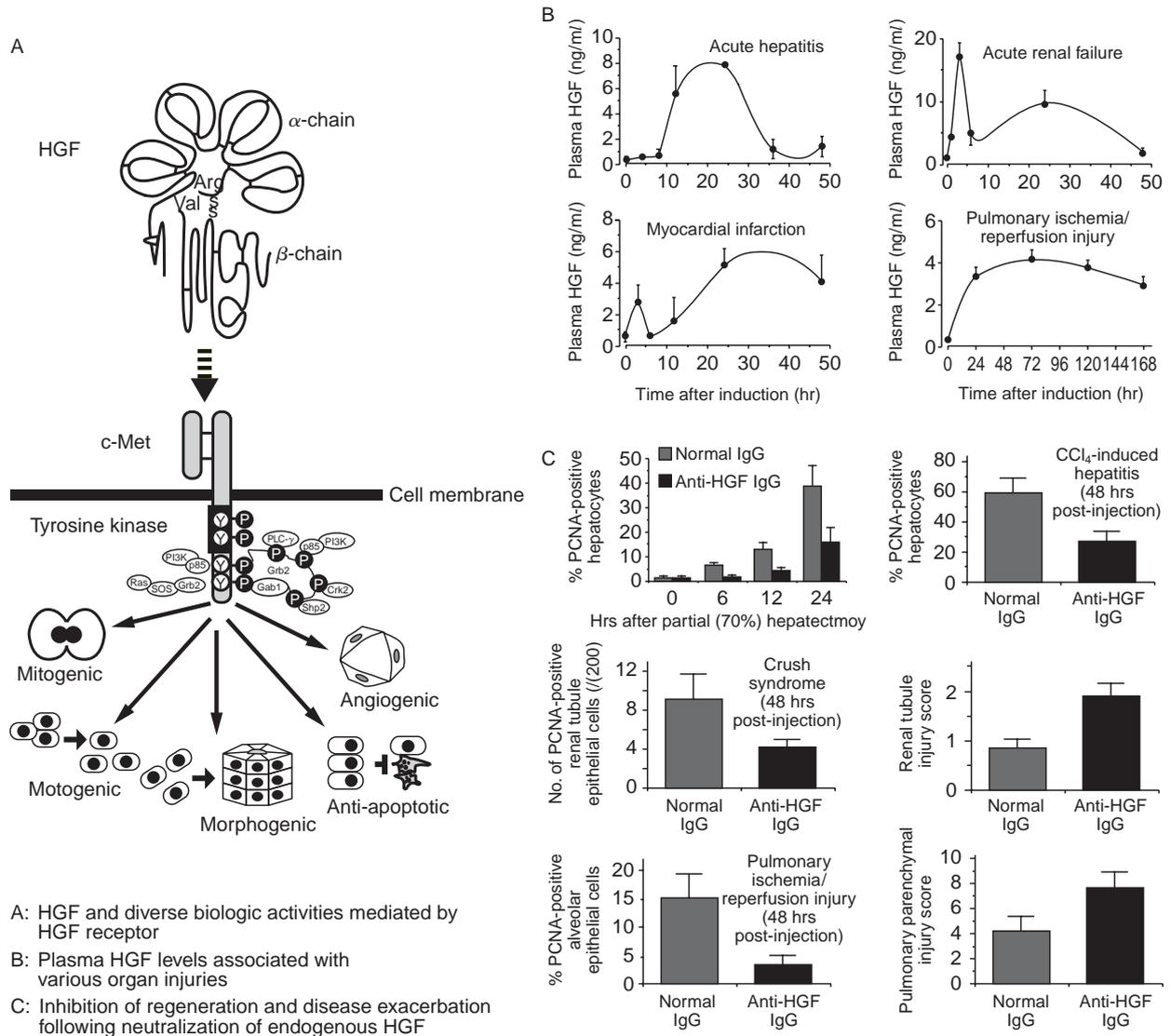


Fig. 1 Biologic activities and roles as an intrinsic regeneration factor of HGF

of the simple duplication system will be a means of regeneration/repair (therapy) in accordance with nature.

This paper discusses regenerative therapy for sclerotic disorders that arise from failure of the simple duplication system's self-repair mechanism due to chronic damage.

### HGF as an Intrinsic Regeneration Factor

Hepatocyte growth factor (HGF) was dis-

covered in 1984 as a long-sought-after hepatotrophic factor for vigorous regeneration of the liver, and its structure was clarified by molecular cloning in 1989.<sup>1)</sup> HGF stimulates proliferation of hepatocytes and practically all types of epithelial cells, as well as some of mesenchymal lineage such as vascular endothelial cells. In 1991, the receptor for HGF was identified to be a tyrosine kinase type c-Met gene product. Ensuing studies have revealed that HGF is endowed not only with the ability to enhance growth of the said target cells (mitogen) but also

has other biologic activities such as enhancement of cellular motility (motogen), induction of morphogenesis represented by tubular structures (morphogen), and anti-apoptotic and angiogenic potential<sup>2)</sup> (Fig. 1A).

From the rapid increase in HGF level of damaged tissue or circulating plasma occurring in response to visceral injury, it has even been suggested that HGF acts as an intrinsic regeneration factor upon various organs (Fig. 1B). Blockade of the injury-associated HGF elevation by an anti-HGF neutralizing antibody results in strong suppression of organ regeneration/repair and expansion of tissue damage (i.e., aggravation of disease state) (Fig. 1C). It is obvious also from these events that HGF acts as an intrinsic regeneration factor to promote post-injury regeneration/repair. Conversely, administration of recombinant HGF to mice after onset of induced acute hepatitis, acute renal failure, myocardial infarction, or pneumonia leads to a conspicuous inhibition of tissue damage (anti-apoptotic effect) and enhanced regeneration with consequent marked improvement.<sup>2,3)</sup>

We then investigated the site(s) of origin of HGF, which is promptly generated and supplied in response to tissue injury. Our results showed that, in case of liver damage, for example, HGF mRNA expression occurs immediately in mesenchymal tissues such as vascular endothelial cells, Kupffer cells, and Itoh cells of the damaged liver and that HGF expression is induced also in such distant intact viscera as the lung, spleen, and kidney. Thus, HGF elaborated upon recognition of an injury assumes physiologic roles for regeneration/repair of the damaged organ via both endocrine and paracrine pathways.

### Regenerative Therapy for Chronic Fibrotic Disorders

In chronic disorders involving persistent tissue damage, destruction of parenchymal cells and loss of vascular endothelial cells that serve

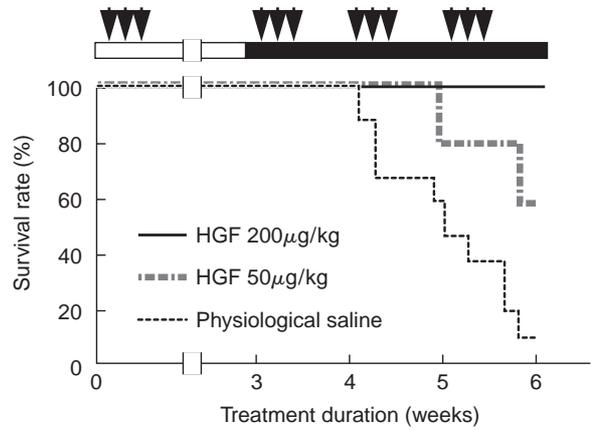
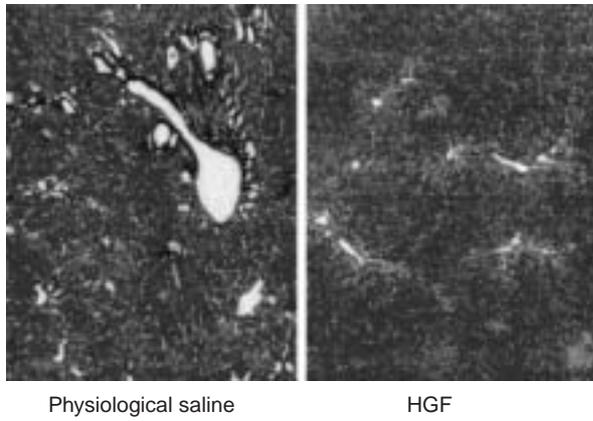
organ functions give rise to proliferation of fibroblasts and other interstitial cells and accumulation of interstitial fibrous components which those cells produce, eventually resulting in sclerosis and dysfunction of the organ. These studies have indicated that decrease in expression of the intrinsic regeneration factor HGF occurs with the progression of chronic disease, coupled reciprocally with overexpression of the transforming growth factor (TGF)- $\beta$ .

TGF- $\beta$  acts as an inhibitor of growth of epithelial cells and vascular endothelial cells and, at the same time, as a promoter for apoptosis. Toward interstitial cells, on the other hand, TGF- $\beta$  functions as a growth-promoting factor and stimulates production of interstitial fibrous components such as collagen. Overexpression of TGF- $\beta$  is thus a major cause of dysregeneration and parallel stimulation of tissue fibrosis.

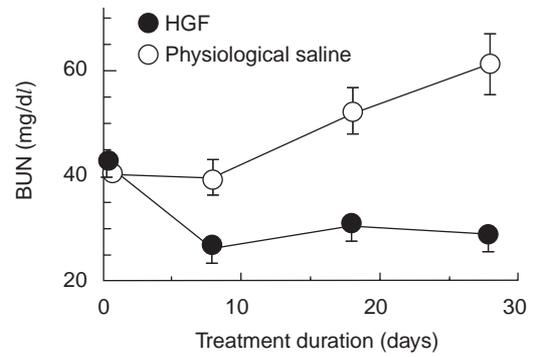
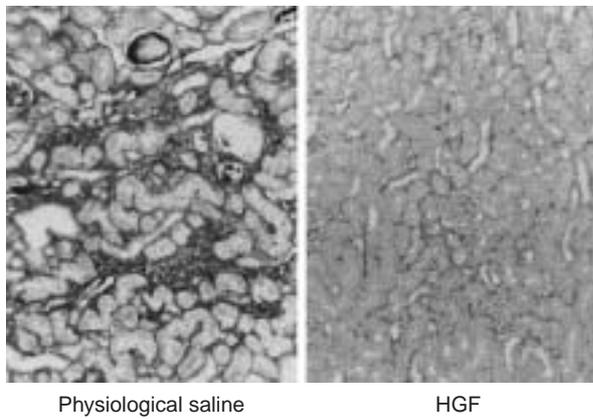
First, we would like to introduce findings demonstrating HGF's ability to inhibit the development of liver cirrhosis and treat cirrhosis. In Japan alone, more than 20,000 patients develop liver cirrhosis due to chronic hepatitis or congenital biliary atresia annually. Liver transplantation is the only life-saving procedure currently available, however, registered donors are extremely few and most cirrhosis patients on the waiting list lose their lives.

Long-term administration of very low doses of dimethylnitrosamine (DMN), a hepatotoxic substance, to rats led to overt development of hepatic fibrosis and in due course to cirrhosis. Deaths began to occur among the animals in Week 4 of DMN dosing and practically all animals succumbed to hepatic failure by Week 6. When rats were started on daily recombinant HGF dosing at Week 3 of DMN administration, at which period hepatic fibrosis had developed, subsequent development of cirrhosis was almost completely inhibited along with amelioration in preexisting hepatic fibrosis and reconstruction of the normal lobular architecture (Fig. 2A).<sup>4)</sup> Thus, the survival rate of rats increased with increasing dosages of HGF;

A: Cirrhosis (Masson's trichrome stain)



B: Chronic renal failure (Immunostain for type I collagen)



C: Pulmonary fibrosis (elastica-Masson stain)

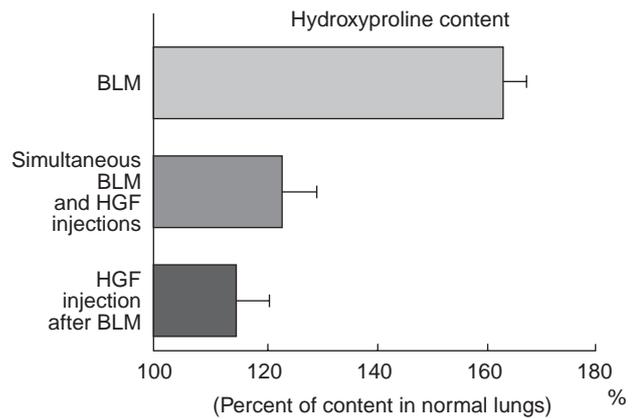
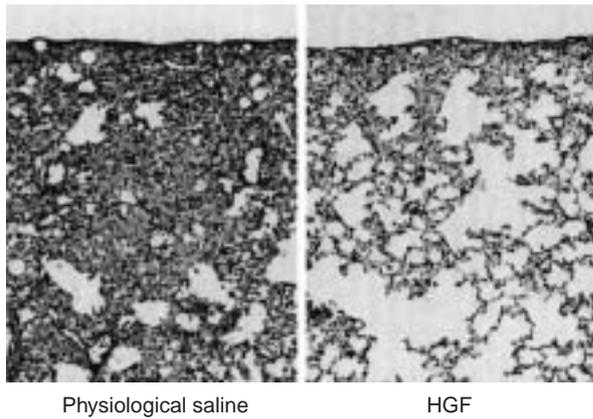


Fig. 2 Therapeutic effects of HGF in fibrous sclerotic disorders

Source: A: Matsuda, Y. *et al.*: *J Biochem* 1995; 118: 643–649.

B: Mizuno, S. *et al.*: *J Clin Invest* 1998; 101: 1827–1834.

C: Yaekashiwa, M. *et al.*: *Am J Respir Crit Care Med* 1997; 156: 1937–1944.

all rats receiving HGF at 200µg/kg b.w. survived in normal health without incurring he-

patic failure.

In rats of the HGF-treated groups, there was

evidence of enhanced growth (regeneration) of hepatocytes, increased collagenolysis, inhibition of TGF- $\beta$  expression as well as hepatocytic apoptosis, along with suppressed extracellular matrix production; the treated animals showed nearly normal histological features of liver tissue architecture.<sup>4)</sup> Gene therapy with HGF-expressed plasmid produced similar therapeutic effects in animals with liver cirrhosis.<sup>5)</sup>

Next, we would like to discuss the therapeutic effects of HGF in chronic renal failure/nephrosclerosis. ICGN mice, being animal models for chronic nephropathies in humans, show gradual impairment of renal function beginning just after 10 weeks of age, develop marked glomerular and tubulointerstitial fibrosis at 18 weeks of age and after, and eventually develop end-stage renal failure. The renal tissue TGF- $\beta$  level increases remarkably at 10 weeks of age and thereafter, and the TGF- $\beta$  elevation is accompanied by accumulation of fibrous components in renal tissues and increased apoptosis of renal tubule epithelial cells and glomerular vascular endothelial cells.

The renal tissue HGF level is decreased, in contrast to the TGF- $\beta$  elevation, so that regenerative capacity of renal tubule epithelial cells is noticeably depressed. Depressed expression of HGF, which is to act as an intrinsic regeneration factor and elevated expression of TGF- $\beta$ , is considered to be etiologically largely responsible for the development and progression of chronic nephropathies. In ICGN mice receiving daily doses of recombinant HGF for 4 weeks starting at 14 weeks of age, the renal function indicator BUN level, which had been continuously increasing, was largely restored to normal 7 days after the start of dosing and remained at normal levels thereafter (Fig. 2B).<sup>6)</sup>

In the HGF-treated groups of mice, a pronounced suppression of TGF- $\beta$  expression in kidney tissues, inhibited accumulation of collagen and other fibrous components, suppressed apoptosis of renal tubule epithelial cells, and a marked enhancement of renal tubule epithelial cells were noted. Consequently, their kidneys

showed histological features close to normal renal tissues, indicating that HGF effected glomerular and renal tubule tissue reconstruction, as seen in Fig. 2B.<sup>6)</sup> Similar results were reproduced in such other chronic renal failure models as chronic kidney graft rejection, hydro-nephrosis, and 5/6-nephrectomized rats.<sup>7)</sup> If reconstruction of renal tissues could be induced by administration of HGF, thereby liberating chronic renal failure patients from hemodialysis therapy, it would be of great medical and economic significance.

The antifibrotic effect has been demonstrated following treatment with HGF in a mouse model for pulmonary fibrosis induced with bleomycin (BLM) injections (Fig. 2C).<sup>8)</sup> We would like to omit the details of the data here on account of limited space. The conspicuous efficacy of HGF lies in regenerating normal tissue architectures as well as in inhibiting apoptosis of parenchymal cells accountable for functions of tissues and organs and in stimulating growth of those cells.

HGF is also an angiogenetic factor with potent ability to promote angiogenesis essential to tissue regeneration/repair. In chronic disorders in particular, depressed HGF expression occurs and eventually leads to elevated expression of fibrosis-promoting factors such as TGF- $\beta$ . This, in turn, accelerates fibrosis via loss and inhibited regeneration of parenchymal tissues. Administration of HGF to subjects in such conditions enhances regeneration of parenchymal cells and vascular endothelial cells and suppresses TGF- $\beta$  expression, with consequent inhibition of tissue destruction and fibrosis.

## Conclusion

This article has briefly described the regenerative therapy of chronic fibrotic disorders for which there has been no definitive treatment by vigorous regeneration/repair *in vivo* using the intrinsic regeneration factor HGF, which is considered to play a crucial role in the simple

duplication system for regeneration/repair inherent in mammals. We feel that there is a fair prospect that regenerative medical care using a recombinant HGF protein or HGF gene will be a concrete therapy that will remove the suffering and promote the welfare of countless diseased individuals.

#### REFERENCES

- 1) Nakamura, T., Nishizawa, T., Hagiya, M. *et al.*: Molecular cloning and expression of human hepatocyte growth factor. *Nature* 1989; 342: 440–443.
- 2) Funakoshi, H., and Nakamura, T.: Hepatocyte growth factor: from diagnosis to clinical applications. *Clin Chim Acta* 2003; 327: 1–23.
- 3) Matsumoto, K. and Nakamura, T.: Hepatocyte growth factor (HGF) as a tissue organizer for organogenesis and regeneration. *Biochem Biophys Res Commun* 1997; 239: 639–644.
- 4) Matsuda, Y., Matsumoto, K., Ichida, T. and Nakamura, T.: Hepatocyte growth factor suppresses the onset of liver cirrhosis and abrogates lethal hepatic dysfunction in rats. *J Biochem* 1995; 118: 643–649.
- 5) Ueki, T., Kaneda, Y., Tsutsui, H. *et al.*: Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nat Med* 1999; 5: 226–230.
- 6) Mizuno, S., Kurosawa, T., Matsumoto, K. *et al.*: Hepatocyte growth factor prevents renal fibrosis and dysfunction in a mouse model of chronic renal disease. *J Clin Invest* 1998; 101: 1827–1834.
- 7) Matsumoto, K. and Nakamura, T.: Hepatocyte growth factor: renotropic role and potential therapeutics for renal diseases. *Kidney Int* 2001; 59: 2023–2038.
- 8) Yaekashiwa, M., Nakayama, S., Ohnuma, K. *et al.*: Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic changes in murine lung injury induced by bleomycin. *Am J Respir Crit Care Med* 1997; 156: 1937–1944.

# Regenerative Medicine for Cardiomyocytes

JMAJ 47(7): 328–332, 2004

Keiichi FUKUDA

*Assistant Professor, Institute for Advanced Cardiac Therapeutics,  
Keio University School of Medicine*

**Abstract:** Heart transplantation is the ultimate treatment option for severe cardiac failure, but is available only for a very small fraction of cases due to a serious donor shortage. Increasing attention has become focused upon a novel therapeutic approach, regenerative medicine, to break the present impasse. Attempts to regenerate cardiomyocytes have been made by using pluripotent embryonic stem cells or marrow-derived mesenchymal stem cells (adult stem cells). Cardiomyocytes can be regenerated from embryonic stem cells as well as from adult stem cells, but the regenerated cells differ in characteristics depending on the source stem cells. These two groups of stem cells differ with respect to proliferative potency, pluripotency, method of induction of differentiation into cardiomyocytes, rejection reactions, and tumorigenic potential. Further studies to ascertain which type of stem cell will be more useful and safer for this purpose remain to be carried out. Studies in laboratory animals have reportedly demonstrated improvement of cardiac function through regenerated cardiomyocyte transplantation into the heart, encouraging the hope that a new treatment modality has been found for severe heart failure.

**Key words:** Embryonic stem cell; Adult stem cell; Cardiomyocyte; Regenerative medicine

## Introduction

In Japan, cases of heart disease have consistently been increasing with the aging of the population and the Westernization of the diet. A wide variety of pharmacotherapies has been developed for the treatment of intractable severe heart disease, with proven efficacy, however, heart transplantation is the sole radical

treatment option. There is still no increase in brain-dead donors and heart transplantation is a treatment available only for a very few cases.

To break the present impasse, therefore, a method to treat intractable cardiac failure by regenerating and transplanting cardiomyocytes is being sought. Studies of heart muscle cell regeneration have been making a steady progress, though at the level of laboratory ani-

---

This article is a revised English version of a paper originally published in the Journal of the Japan Medical Association (Vol. 129, No. 3, 2003, pages 365–368).

Table 1 Comparison of ES Cells and Adult Stem Cells as Materials for Regenerative Cardiomyocytes

	ES Cells	Adult Stem Cells
Origin	Post-fertilization early-stage embryo (inner cell mass of blastocyst)	Marrow stromal cell
Cell isolation technique, etc.	Method of cell establishment is already established, and is relatively easily performed.	Sparse among bone marrow cells, and method of cell establishment is yet to be established.
Proliferative potency	At present, cells are considered to infinitely proliferate.	Proliferate to some extent but the number of divisions is unknown.
Pluripotency	Differentiate into any type of cell <i>in vivo</i> . The cells differentiate into early developmental stage cells <i>in vitro</i> , but it is thought to be difficult for them to differentiate into cells that appear late in the fetal stage.	Recognized to be able to differentiate into mesodermal cells such as osteoblasts, chondroblasts and adipocytes, but reportedly undergo differentiation into nerve cells (ectoblast-derived) and cells of the liver (entoblast-derived) as well.
Differentiation into cardiomyocytes	Differentiate relatively easily, but a method to have ES cells specifically differentiate into cardiomyocytes has not been established.	Demonstrated to differentiate into cardiomyocytes, but a method to have the cells specifically differentiate into cardiomyocytes has not been established.
Rejection reactions	Occur	No rejection reactions if the cells are autologous.
Tumorigenic potential	There is potential risk of teratomas after transplantation if undifferentiated cells remain.	No

mal experiments, and will lead to development of research with human cells and their clinical application.

Stem cells currently used for regenerative medical therapy for the myocardium are broadly divided into two groups: embryonic stem cells (ES cells) obtained from early stage embryos post *in vitro* fertilization and marrow adult stem cells obtained from the bone marrow of adults. Whether one type is superior to the other for cardiomyocyte regeneration remains to be seen. Characteristics of the two types of stem cells are summarized in Table 1. This article outlines the current status and future prospects of regenerative medicine for the myocardium.

### Differentiation from ES Cells to Cardiomyocytes

Figure 1 illustrates the outline of cardiomyocyte regeneration using ES cells. ES cells are those cells constituting the inner cell mass, destined to form the fetus, from an early

embryo having reached the stage of blastocyst. These cells are known to differentiate into any type of cell *in vivo* and have been shown to differentiate *in vitro* into a variety of organic cells such as cardiomyocytes, skeletal muscle cells, vascular endothelial cells, smooth muscle cells, neurons, and hepatocytes. However, most types of cells that appear late in the fetal stage have not been demonstrated from ES cells *in vitro*.

It is generally recognized that various cell growth factors, cytokines, and cell adhesion factors are required for ES cell differentiation into those various cells. Recent studies have clarified a cascade operating for selective differentiation of ES cells into motor neurons, however, the cascade for ES cell differentiation into cardiomyocytes has not been fully elucidated to date.

A method to have ES cells form a cell mass (embryoid) has been introduced as a general means of inducing ES cell differentiation into cells capable of differentiation. The frequency with which differentiation from the embryoid

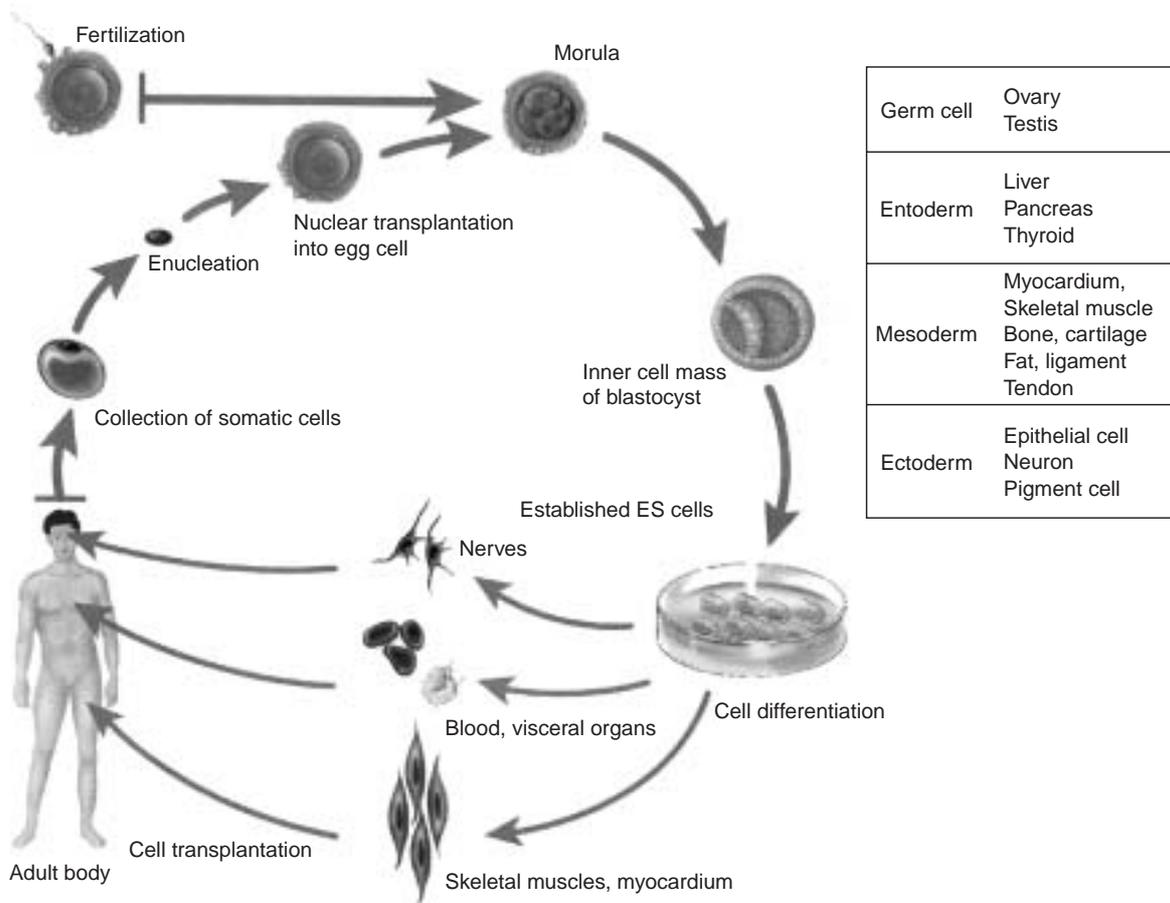


Fig. 1 Myocardial regeneration from ES cells

to cardiomyocytes is regarded as being about 7–8% at best. Humoral factors known to induce ES cell differentiation specifically into cardiomyocytes include bone morphogenetic protein-2 (BMP-2) and Wnt 11, which facilitate the differentiation, and Wnt 3 and Wnt 8, which act as inhibitors. Possible involvement of various other factors is presumed; the process seems to be quite intricate. Selective differentiation of ES cells into cardiomyocytes may become feasible from analysis of the pathways of the differentiation process.

### Adult Stem Cell Differentiation into Cardiomyocytes

Bone marrow has been universally recognized to be the site of hematopoiesis with the

predominance of hematopoietic stem cells. In fact, more than 99% of marrow cells take part in the production and development of blood cells. It was discovered recently that, among bone marrow cells, there are cells termed stromal cells that essentially do not represent blood cells but secrete cytokines and growth factors to support cells of the hematopoietic system. The presence of pluripotent stem cells capable of differentiating into various types of cells among the marrow stromal cells has become recognized. These marrow stromal cells with pluripotent capacity are referred to as mesenchymal stem cells on account of their ability to differentiate into such mesenchymal cells as osteoblasts, chondroblasts, and adipocytes.

In view of the mesenchymal stem cells being

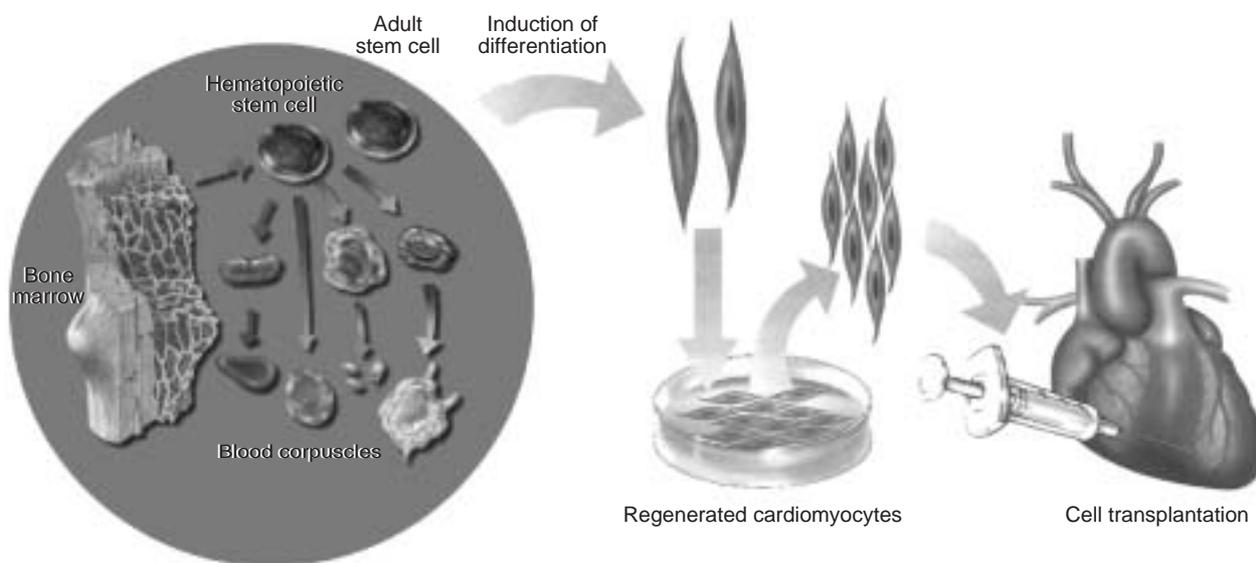


Fig. 2 Myocardial regeneration from adult stem cells and cell transplantation

able to differentiate into mesoblast-derived organs, we wondered if they could differentiate to become cardiomyocytes as well, which are also of mesodermal origin. Our studies demonstrated that cardiomyocytes that beat regularly by themselves can be obtained from mesenchymal stem cells. Figure 2 shows an outline of the process. Mesenchymal stem cells have recently been reported to undergo differentiation into nerve cells (ectoblast-derived) and cells of the liver (entoblast-derived) as well, and are now termed adult stem cells.

### Characteristics of Regenerated Cardiomyocytes

Cardiomyocytes derived from bone marrow show expression of fetal ventricular muscle type genes soon after their differentiation from adult stem cells, and thereafter gradually express adult type genes.<sup>1)</sup> Expression of genes for atrial natriuretic polypeptide and cerebral natriuretic polypeptide that are considered cardiomyocyte-specific have also been demonstrated. The cells proved to self-beat, and those differentiated from murine adult stem cells showed 120–250 beats/min. The cardiomyo-

cytes exhibited the sinus node pattern of action potentials early after their differentiation from adult stem cells, and the pattern gradually changed to the ventricular muscle cell type.

Catecholamin  $\alpha_1$  receptor (cardiac hypertrophic effect) and  $\beta_1$  and  $\beta_2$  receptors (positive chronotropic and positive inotropic effects) play important roles in the muscle cells of the heart. In regenerated cardiomyocytes of bone marrow origin,  $\alpha_1$  receptor switched to myocardial type (mainly  $\beta_{1A}$  and  $\beta_{1B}$  receptors) as the differentiation proceeded into heart muscle cells, and simultaneously,  $\beta_1$  and  $\beta_2$  receptors that had not existed early in the course of differentiation became expressed.<sup>2)</sup> Stimulation of these catecholamine receptors led to activation of subreceptor signaling to produce cardiac hypertrophy, increases in heart rate and increases in myocardial contractile force.

The above findings indicate that the regenerated cardiomyocytes are endowed with practically normal characteristics of cardiomyocytes.<sup>3)</sup>

### Treatment of Cardiac Failure by Cell Transplantation

Cardiomyocyte transplantation has been

extensively investigated since the mid-1990s at the level of animal experiments. Cells harvested in primary cultures of heart muscle cells obtained from the fetus or the neonate were transplanted into the heart of sexually mature animals, and the transplantation was shown to improve post-infarction cardiac function. Clinical experience with the transplantation of fetal midbrain obtained through artificial termination of pregnancy into patients with Parkinson's disease has yielded some gratifying therapeutic results. The amount of cell transplants required is apparently greater in the case of cardiomyocyte transplantation, so that it is not practical to use aborted fetuses as the source.

Regenerated cardiomyocytes derived from ES cells, when transplanted, reportedly proved to electrically bound to recipient myocardium and contract synchronously with surrounding cells, thus fueling hopes for the use of regenerated cardiomyocytes. Our experience with regenerated cardiomyocyte transplantation into the hearts of adult patients showed long-term engraftment with gratifying outcomes. However, there have been reports demonstrating that the number of cardiomyocytes taken as compared to that of cardiomyocytes transplanted diminished due to cellular necrosis during the course of engraftment. Further study is needed, including assessments of transplantation methods.

### Problems Associated with Myocardial Regeneration and Future Prospects

To bring regenerative cardiomyocyte trans-

plantation to realization requires securing regenerated cardiomyocytes and supplying those cells safely and at moderate expense. When adult stem cells and ES cells are compared, this author's view is that the latter will come into use earlier in the future. Supply of regenerated cardiomyocytes derived from ES cells will become a reality within several years. Major problems are rejection reactions and method of transplantation. To avoid rejection, it is essential to transplant the nucleus of a somatic cell into an egg cell, as shown in Fig. 1. The nuclear transplantation eventually has a close bearing upon the matter of human cloning, therefore it is important to hold nationwide discussion of this ethical problem.

### REFERENCES

- 1) Makino, S., Fukuda, K., Miyoshi, S. *et al.*: Cardiomyocytes can be generated from marrow stromal cells *in vitro*. *J Clin Invest* 1999; 103: 687–705.
- 2) Hakuno, D., Fukuda, K., Makino, S. *et al.*: Bone marrow-derived regenerated cardiomyocytes (CMG Cells) express functional adrenergic and muscarinic receptors. *Circulation* 2002; 106: 380–386.
- 3) Fukuda, K.: Differentiation from bone marrow mesenchymal stem cells into cardiomyocytes. *In*: Asajima, M., Iwata, H., Ueda, M. and Nakatsuji, N. (Eds.): "Regenerative medicine and life science — Reproductive engineering, stem cell engineering and tissue engineering", *Protein, Nucleic Acids and Enzymes*, (Special Issue), Kyoritsu Shuppan, Tokyo, 2000; 45 (13): 2078–2084. (in Japanese)

# Regenerative Medicine for Respiratory Diseases

JMAJ 47(7): 333–337, 2004

Tatsuo NAKAMURA

*Associate Professor, Institute for Frontier Medical Sciences, Kyoto University*

**Abstract:** Tissue engineering is an important technology for supporting regenerative medicine, producing tissues in incubation bottles in a tissue culture laboratory. In addition, a newer approach to regeneration of tissues in the human body, called *in situ* tissue engineering, has been devised in Japan and is attracting attention as a new means of bringing out our body's potential. New therapeutic methods for chronic respiratory disorders such as pulmonary emphysema and pulmonary fibrosis, as well as for primary pulmonary hypertension for which there have been no curative treatment measures available, are currently under investigation. This paper provides a full account of the developments. *In situ* tissue engineering causes regeneration of the lung parenchyma itself within the lungs using various growth factors and collagen, which serves as a scaffold for tissue regeneration. A new type of tracheal prosthesis has been in clinical use since 2002 in which new technique was applied to induce autologous tissue regeneration.

**Key words:** Tissue engineering; Artificial trachea; *In situ* tissue engineering; Primary pulmonary hypertension

## Introduction

The number of patients suffering from dyspnea after surgical resection of carcinoma of the lungs and patients with chronic respiratory disorders such as pulmonary emphysema or pulmonary fibrosis is expected to increase continuously in the future. Primary pulmonary hypertension is also a progressive disease, though lower in incidence, with an extremely poor prognosis. This paper reviews the present

state of regenerative medical care for these respiratory disorders, with particular reference to the progress of research aimed at application in clinical settings.

## Artificial Trachea (Bronchus)

Obstructive disorders and stenosis of the central airway are mainly caused by infiltration of lung cancer or thyroid carcinoma. These disorders are rarely indicated for surgery because

---

This article is a revised English version of a paper originally published in the Journal of the Japan Medical Association (Vol. 129, No. 3, 2003, pages 369–372).

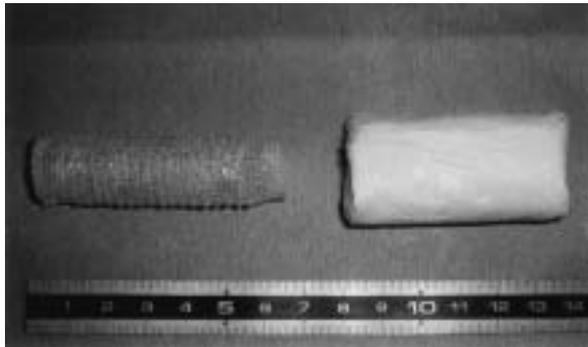


Fig. 1 Autologous tissue regeneration type tracheal prosthesis

A structural equivalent of tracheal cartilage comprising a polypropylene stent spirally wrapped around a Marlex mesh (left) and coated with collagen sponge. Autologous tissue regenerates in this collagen scaffold layer.

of lack of reconstructive procedures, despite an extensive resection of the malignancies. Development of an artificial trachea that can be used safely even in such patients has already begun in early 1940s. Initially, a variety of attempts were made to replace the affected trachea with an artificial tube like a vascular prosthesis, but all proved unsuccessful. Artificial tracheas were then developed using the new idea of having autologous tissue regenerate on the surface of a tracheal prosthesis. This artificial trachea is composed of a Marlex mesh tube reinforced by a spiral stent and is conjugated with collagen that has a good affinity for tissues (Fig. 1).

Okumura *et al.* were the first to succeed in the use of this artificial trachea for regeneration of a 5-cm canine cervical trachea.<sup>1)</sup> The host's tissues replace the collagen on the implanted artificial trachea while the mesh and stent integrate as being embedded with the host's tissues. Further, the epithelium spreads from the stumps, and eventually, continuously covers the luminal surface. The use of a Y-shaped collagen-conjugated prosthesis was demonstrated to be safe and effective in the reconstruction of the carina tracheae, which is the most difficult operation in the surgery of respiratory organs.<sup>2)</sup> The long-term safety of carinal reconstruction has been con-

firmed over a 5 years observation.<sup>3)</sup> This tissue regeneration-type tracheal prosthesis is now clinically used as a trial upon an ongoing ethical committee review.

### Regeneration of Pulmonary Parenchyma and *In Situ* Tissue Engineering

Regeneration of the lungs, unlike the liver, has not been recognized. However, the recent advances in regenerative medicine have shown that the lungs can also be regenerated. Regeneration of destroyed pulmonary parenchyma, if feasible, will enable treatment of disorders for which there has long been no curative treatment, such as pulmonary emphysema, pulmonary fibrosis, and pulmonary hypertension.

A group of investigators at Harvard Medical School seeded the lung cells obtained from a sheep that had been trypsinized on biodegradable polyglycolic acid (PGA) fiber mesh and incubated with added growth factors such as epithelial cell growth factor (EGF) and basic fibroblast growth factor (bFGF). The investigators implanted the cells on fiber mesh in the subcutis of nude mice. One week later, the implant was noted to have become a tissue resembling lung tissue capable of producing collagen and surfactants. At 2 weeks after the implantation, an extracellular matrix with an alveolar structure (i.e., type I and type II alveolar epithelium and bronchioid patterns) had formed.<sup>4)</sup> Thus, even the lungs can be formed if pulmonary cells are used under certain conditions.

Apart from such attempts, studies aimed at regeneration of new lung tissues with a scaffold placed in the lungs have been conducted by Itoi *et al.* in Japan.<sup>5)</sup> When a collagen sponge, which serves as a scaffold for regeneration, is implanted in the lung of a rabbit, the implanted collagen is gradually degraded and absorbed and cells infiltrate it. On Day 5 after the operation, tubular structures had formed in the border of the surrounding lung tissue and had

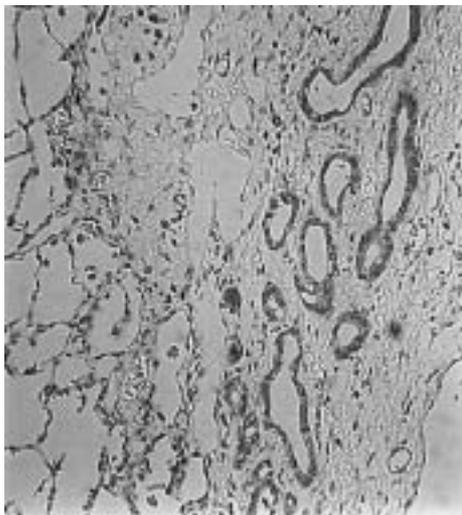


Fig. 2 Tissue regenerated in the collagen sponge scaffold implanted in the lung

(Courtesy of Dr. S. Itoi, Institute for Frontier Medical Sciences, Kyoto University)

Note the regenerated duct cells forming tubular structures continuous to adjacent normal regions. Photomicrograph at 5 weeks after implantation. Normal lung tissue at left. Lung tissue can be regenerated with the new tissue engineering technique.

joined with normal lung tissue (Fig. 2). The changes move in due course to the central portion of the implant, so that the implanted collagen scaffold becomes replaced by newly regenerated lung tissue. The cells showing tubular structures are considered to be of Clara cell or type II alveolar cell origin from the results of the immunostaining examination.

The method of regenerating tissue *in situ* with a scaffold placed in the body is referred to as *in situ* tissue engineering, and there is growing hope for its clinical application. In addition, development of treatment methods to repair and regenerate lung tissues using growth factors and cells is also in progress as described below.

### Pulmonary Fibrosis

It is now generally recognized that pulmonary fibrosis arises from progressive lung remodeling caused by proliferating extracel-

lular matrix due to inflammatory cells. The strategy of treatment has been mainly suppression of inflammation, but recently, progress has been made in the areas of tissue repair and regeneration.

The pathogenesis of pulmonary fibrosis are implicated in cytokines, such as fibroblast growth factor (FGF) and transforming growth factor (TGF)- $\beta$ , which are elaborated by inflammatory cells. Meanwhile, hepatocyte growth factor (HGF) inhibits the effects of these cytokines; intrapulmonary collagen levels are maintained via equilibrium between these factors. Degradation of extracellular matrix is effected by matrix metalloproteinase (MMP) and other proteases, and it has been demonstrated that the tissue level of MMP is elevated in patients with pulmonary fibrosis. In view of this, research in regenerative medicine is currently in progress to regenerate a normal lung from fibrotic pulmonary structure by effective intrapulmonary administration of HGF and other cytokines and MMP.<sup>6)</sup>

### Pulmonary Emphysema

The number of the patients of pulmonary emphysema has been rising progressively; the disease is the third or fourth leading cause of death in Western countries. Corticosteroids, expectorants and bronchodilators prescribed for the treatment of pulmonary emphysema are aimed at suppressing the progression of the disease.

Pulmonary emphysema is known to progress due to imbalance between proteases and antiproteases in lung tissues. Accordingly, administration of such growth factors as HGF, EGF and TGF- $\beta$ , use of protease inhibitors to elastase, MMP and cathepsin, and use of inhibitors to all-trans retinoic acid (ATRA) and other neutrophil activators are being assessed as a drastic therapy.<sup>7)</sup> Medication with these substances represents an attempt to regenerate pulmonary tissues by stimulating local stem cells that may remain in the

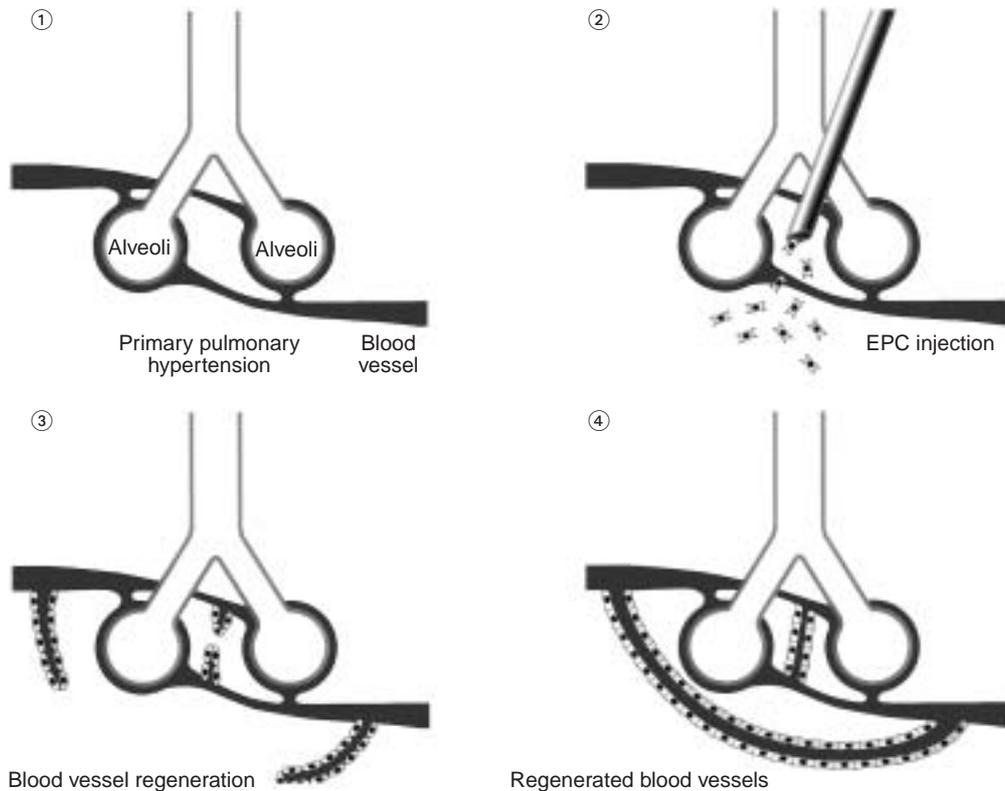


Fig. 3 Shema showing the new treatment method for primary pulmonary hypertension. Endothelial progenitor cells (EPC) collected from autologous peripheral blood and grown *in vitro* are injected directly into the lung to allow neoangiogenesis for improvement of pulmonary hypertension.

damaged lung.

Studies in this field had been confined to laboratory investigations using small rodents, therefore, there is a wide gap from the situation in clinical settings. Toba *et al.* demonstrated regeneration from emphysematous lung tissues with an improvement in pulmonary function by trans-bronchoscopic administration of TGF- $\beta$  in a canine (beagle) model of pulmonary emphysema induced with intra-airway elastase spraying, thus taking a distinct step toward clinical application.<sup>8)</sup>

### Primary Pulmonary Hypertension (PPH)

Primary pulmonary hypertension is a disease with an extremely poor prognosis that is characterized by incipient symptoms of shortness of breath on exertion and right cardiac failure and

an invariably fatal outcome in 2–10 years (average: 5 years). While lung transplantation is used to treat this disease mainly in Western countries, a novel treatment based on regenerative medicine has been devised and is attracting attention. Endothelial progenitor cells (EPC) concerning angiogenesis have been demonstrated to be present in a peripheral blood cell subpopulation called peripheral blood mononuclear cells (PBMCs). Autologous EPC isolated from the peripheral blood of the patient and grown *in vitro* are injected into the lung to regenerate new blood vessels in the lesion for treatment of PPH (Fig. 3).

Takahashi *et al.* trans-bronchoscopically injected autologous EPC that had been isolated from the peripheral blood and grown *in vitro* into the lung tissue of a canine PPH model. This resulted in regeneration of new blood vessels

in the lung of the dog, which then showed improvement of cardiac function, which had been depressed due to pulmonary hypertension.<sup>9,10)</sup> The pioneering results from Japan have shed a great ray of hope for the treatment of PPH, for which lung transplantation has been the only curative treatment available.

## Conclusion

With the advent of the twenty-first century, regenerative medical care has been steadily applied in clinical settings to respiratory diseases as well. A new treatment modality is thus being developed even for disorders for which no radical treatment has been available.

## REFERENCES

- 1) Okumura, N., Nakamura, T., Natsume, T. *et al.*: Experimental study on a new tracheal prosthesis made from collagen-conjugated mesh. *J Thorac Cardiovasc Surg* 1994; 108: 337–345.
- 2) Sekine, T., Nakamura, T., Matsumoto, K. *et al.*: Carinal reconstruction with a Y-shaped collagen-conjugated prosthesis. *J Thorac Cardiovasc Surg* 2000; 119: 1162–1168.
- 3) Nakamura, T., Teramachi, M., Sekine, T. *et al.*: Artificial trachea and long term follow-up in carinal reconstruction in dogs. *Int J Artif Organs* 2000; 23: 718–724.
- 4) Cortiella, J., Vacanti, C.A. and Vacanti, M.P.: Tissue engineered lung. *Tissue Eng* 2000; 16: 661.
- 5) Itoi, S., Nakamura, T. and Shimizu, Y.: *In situ* tissue engineering for lung regeneration using a collagen sponge scaffold. *ASAIO J* 2001; 47: 173.
- 6) Yaekashiwa, M., Nakayama, S., Nukiwa, T. *et al.*: Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic changes in murine lung injury induced by bleomycin. A morphologic study. *Am J Respir Crit Care Med* 1997; 156: 1937–1944.
- 7) Barnes, P.J.: Novel approaches and targets for treatment of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 160: S72–S79.
- 8) Toba, T., Takahashi, M., Fukuda, M. *et al.*: A study on bronchoscopic preparation of dog models for interstitial pneumonia and emphysematous lungs. *Jpn J Chest Surg* 2002; 16: 473. (in Japanese)
- 9) Takahashi, M., Nakamura, T., Toba, T. *et al.*: New therapeutic option for primary pulmonary hypertension utilizing endothelial progenitor cells. *ASAIO J* 2002; 48: 188.
- 10) Takahashi, M., Nakamura, T. and Katou, H.: Transplantation of endothelial progenitor cells into the lung to alleviate pulmonary hypertension in dogs. *Tissue Engineering* in press.

# Implications of Research Findings Obtained from Centenarians

JMAJ 47(7): 338–343, 2004

Hiroshi SHIBATA

*Professor of Gerontology, Graduate School of Obirin University*

**Abstract:** The number of centenarians in Japan has increased more than a hundred times over the past four decades, reaching nearly 20,000 persons currently. The rate of centenarians in the population is higher in the western part of Japan, where mortality from stroke is low. Centenarians generally have nutritionally well-balanced diets that are rich in animal food and vegetables. They tend to be extroverted, optimistic, hard working, and scrupulous. A recent national survey on centenarians revealed that they have a positive view of their health status, although the degree of independent functioning in activities of daily living is low.

**Key words:** Long life; Lifestyle; Quality of life

## Introduction

According to the author's research, the longest-lived person in Japan has reached the age of 116 years. Although some records of longer-lived individuals have been found, they all tended to be flawed for one reason or another.<sup>1)</sup> Since Japan has an excellent system of census taking, reports of the existence of very long-lived individuals in other societies also are unlikely to be trustworthy. In fact, it was revealed by a US research group that not a single centenarian had been found in Vilcabamba, an Ecuadoran city in South America, which has been considered one of the world's three main areas of longevity.<sup>2)</sup> The

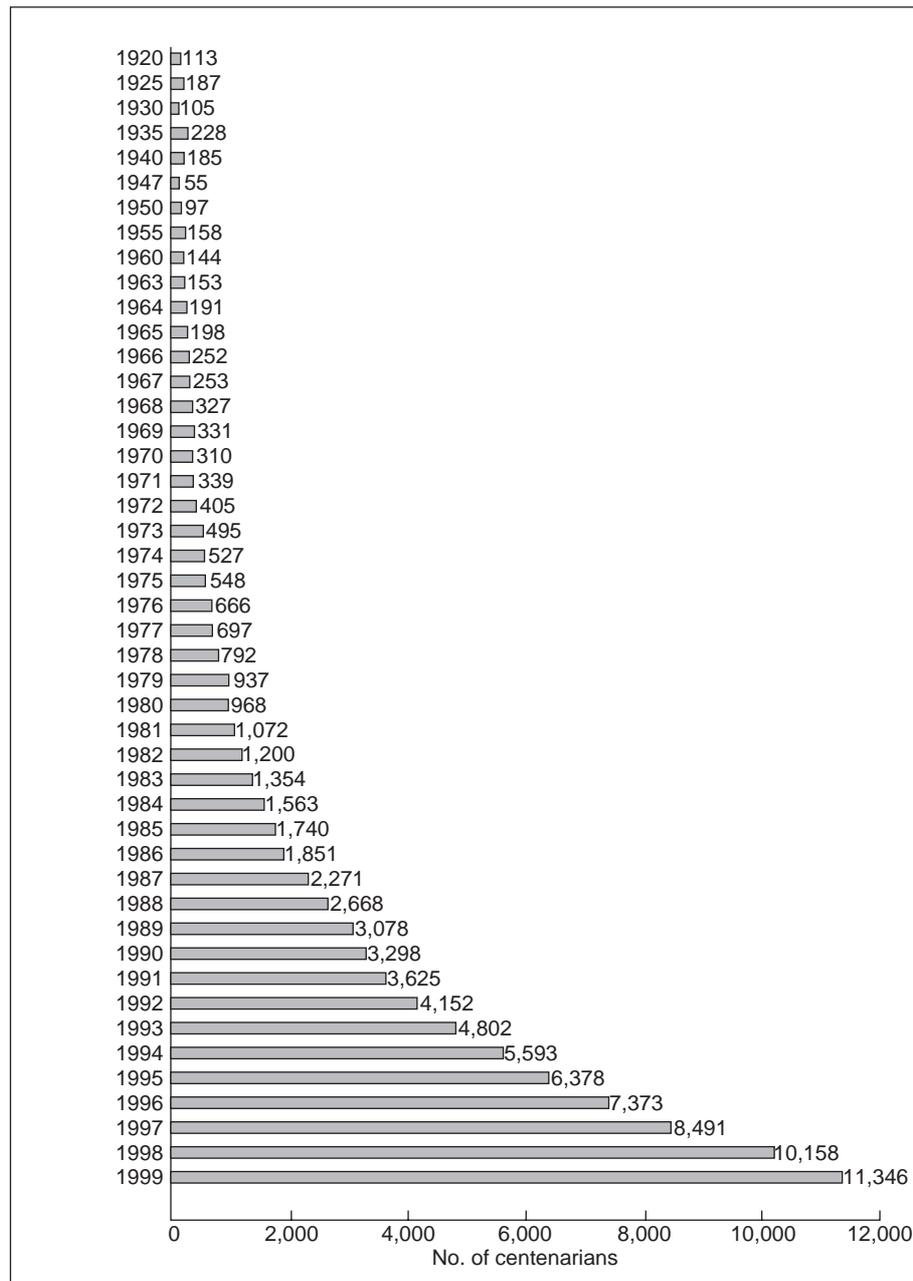
Caucasus, another of the areas famous for its inhabitants' longevity, can also be ruled out, because the demographic statistics of the former Soviet Union were problematic.

Thus, according to reliable statistics, the human life span appears to be less than 120 years. Therefore, centenarians are those who have lived nearly to the limit of the human life span, and their existence provides hope for the longevity of humankind. In Japan, several national surveys on centenarians have been carried out, in addition to various regional surveys. A review of such surveys has been reported by Shinkai, who indicated the direction of future research.<sup>3)</sup>

Most of the previous studies of centenarians

---

This article is a revised English version of a paper originally published in the *Journal of the Japan Medical Association* (Vol. 128, No. 7, 2002, pages 1075–1079). The Japanese text is a transcript of a lecture originally aired on April 15, 2002, by the Nihon Shortwave Broadcasting Co., Ltd., in its regular program "Special Course in Medicine".



Sources: National census (1920–1960) and surveys by the Elderly People's Welfare Division, Social Affairs Bureau, Ministry of Health and Welfare (since 1963).

Fig. 1 Changes in the number of centenarians<sup>7)</sup>

were case studies that attempted to determine factors involved in longevity.<sup>4)</sup> However, the project known as the national cross-sectional study of 1/2 samples of centenarians, which was part of the study on regional systems to aid the elderly in maintaining or improving daily living and to promote participation in social

activities, focused on the quality of centenarians' lives.<sup>5,6)</sup> In this study, which was conducted under the Ministry of Education, Culture, Sports, Science and Technology, the author and other researchers attempted to address the day-to-day needs of the elderly.

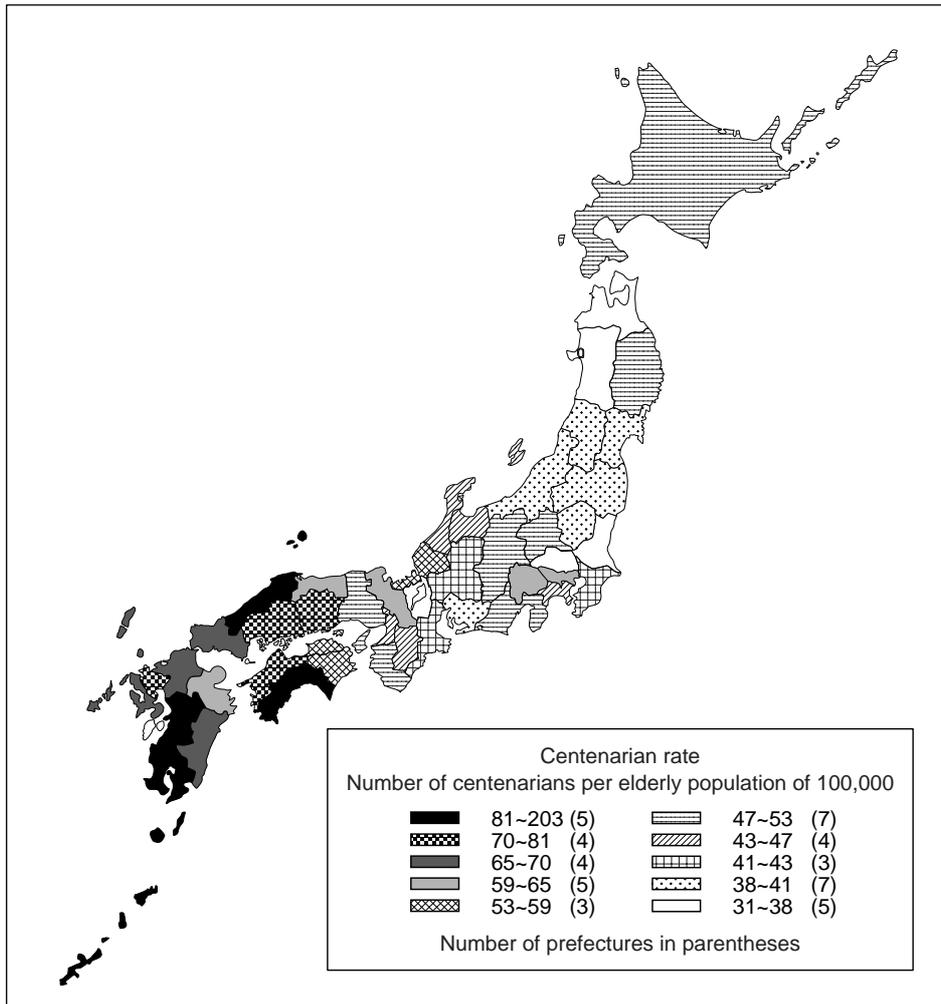


Fig. 2 Centenarian rate by prefecture (1999)<sup>7)</sup>

### Historic Changes and Geographic Distribution of Centenarians

Figure 1 shows changes in the number of centenarians in Japan.<sup>7)</sup> Statistical data on centenarians became more accurate after 1963, when the Old-Age Persons' Welfare Law was enacted on their behalf. The latest data show that centenarians total nearly 20,000 individuals, representing more than a 100-fold increase from 1963. Interestingly, despite this dramatic increase in centenarians, there has been no tendency for the upper age limits to increase, and no one has exceeded 116 years of age. The highest age of centenarians has remained about

110 years, with little change over the years.<sup>1)</sup> This underscores the author's view that the outer margin of longevity for humans is about this level.

The geographic distribution of centenarians in Japan shows that the western part of the country fares better in producing them.<sup>7)</sup> The distribution of centenarians shows an inverse correlation with mortality from stroke, and, in relation to longevity, there is a closer correlation with mean life expectancy at age 65 than that at age 0. When comparing the distribution of centenarians according to prefecture, the rate of centenarians to the total prefectural population is often used, but, for fair compari-

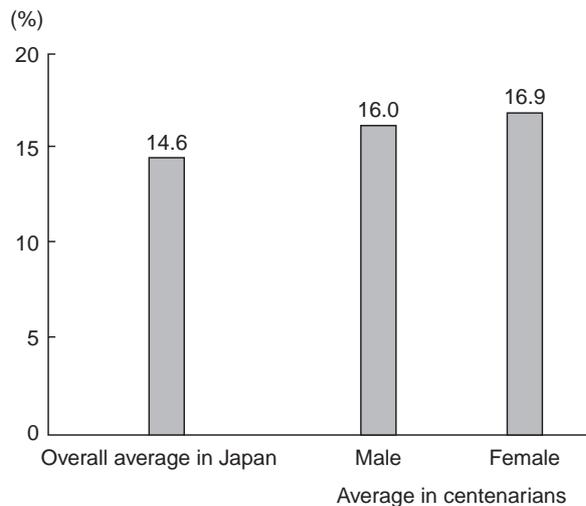


Fig. 3 Comparison of the proportion of protein calories to total calorie intake<sup>4)</sup>

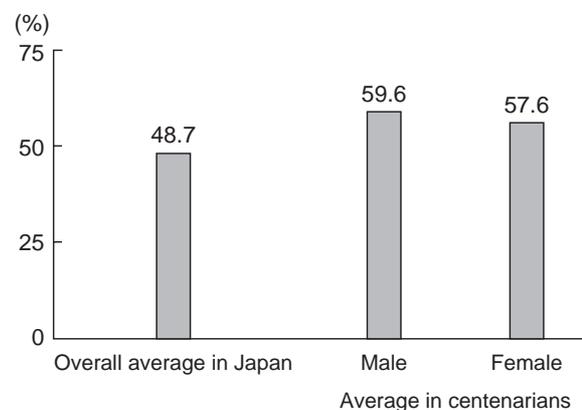


Fig. 4 Comparison of the proportion of animal protein to total protein intake<sup>4)</sup>

son, this measure requires adjustment by the rate of individuals over the age of 65. Therefore, use of the rate of centenarians to the prefectural population of those 65 years old or older is appropriate. Figure 2 shows the rate of centenarians to the population of those 65 years old or older by prefecture in 1999, according to our calculations. The number of centenarians per population of 100,000 aged 65 or older was highest in Okinawa (202.8), followed by Kochi (113.4), Kumamoto (92.7), Shimane (91.4), and Kagoshima (82.7). At the other end of the spectrum, the corresponding rate was lowest in Saitama (31.9), Aomori (32.1), Ibaraki (34.7), Shiga (35.9), and Akita (36.5).

An analysis of factors determining the geographical distribution of centenarians yielded interesting results.<sup>6)</sup> There was a strong correlation between average temperature and the rate of centenarians ( $r=0.740$ ,  $p<0.001$ ). However, the rate of centenarians was higher in Hokkaido, where the average temperature was lowest, than in the entire Tohoku area, indicating that the outside temperature is not necessarily a decisive factor. Although there was an inverse correlation with total calorie intake at 60–69 years of age, it may be premature to

associate calorie restriction with long life, considering the fact that average height is greater in eastern Japan. The proportion of animal protein to total protein intake in persons in their 60s showed a significant positive correlation with the rate of centenarians. However, this index has a geographical distribution similar to that of temperature, so that careful multivariate analysis may be necessary before causality can be discussed.

### Dietary and Lifestyle Habits of Centenarians

Our national survey carried out in 1972 and 1973 provided a detailed inquiry into the eating habits of centenarians for the purpose of nutritional analysis.<sup>4)</sup> Probably because centenarians had a smaller physique and were less active, total daily calorie consumption was 1,073 kcal for males and 939 kcal for females, about half the corresponding totals for the general Japanese population. Data on the overall Japanese population were derived from the 1972 National Dietary Survey. Interestingly, as shown in Fig. 3, the rate of calories derived from protein to total calories was higher in centenarians than in the general population.

Namely, they consumed a low-calorie, high-protein diet. More surprisingly, the proportion of animal protein to total protein intake in centenarians was much higher than that in the general population (Fig. 4). These data indicated a diet almost opposite to a vegetarian diet. When data collection was limited to centenarians with good memory function, we found that their eating habits before the war were similar to those of the general population, indicating that these centenarians altered their eating habits after reaching their middle 70s, almost as if they took the lead in the westernization of Japan's eating habits.

The tendency of centenarians to adopt positive, well-balanced eating habits has also been demonstrated by survey research conducted by the Japan Health Promotion & Fitness Foundation.<sup>8)</sup>

The 1993 survey by this foundation included the exercise habits of centenarians.<sup>9)</sup> The centenarians lived through the era dominated by the nation's policy of increasing wealth and military power, and their occupational life chiefly consisted of physical labor. Therefore, it is presumed that they began to exercise for pleasure only after retirement. In the 1993 survey, 52% of centenarians replied that they exercised, and 43% of them replied that they exercised daily. Specifically, "walking" was predominant for both men and women, followed by "field labor/weeding/caring for plants", "gymnastic exercise", and "work". Compared with centenarians who had no regular exercise, centenarians who engaged in regular exercise were better in activities of daily living (ADL), showed more interest in hobbies, associated more actively with friends, and had eating habits that were improved from those in middle age.

According to our most recent survey on centenarians, 63.8% of male centenarians and 93.2% of female centenarians were never smokers. However, 30.7% of males and 6% of females were past smokers, and 5.6% of males and 0.8% of females were current smokers.<sup>6)</sup>

With regard to alcohol consumption, 45% of males and 77.6% of females were never drinkers, whereas 31.1% of males and 14.9% of females were past drinkers, and 21.9% of males and 5.8% of females were current drinkers.

Thus, habitual drinking was more common than habitual smoking, consistent with data from previous studies on long life in various parts of the world.

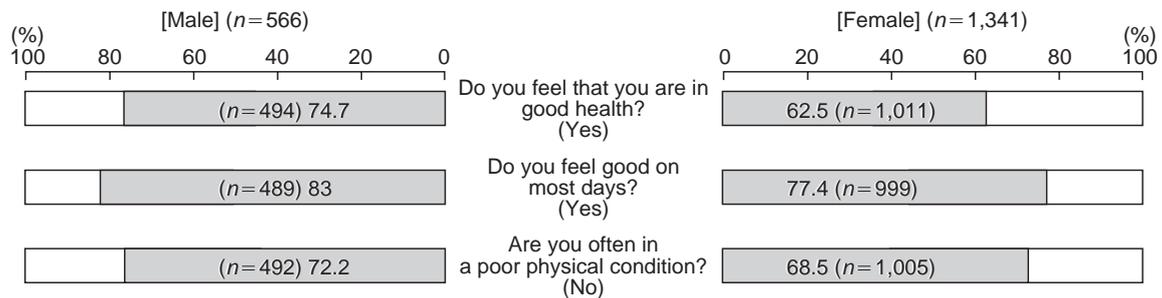
### Characteristics and Personalities of Centenarians

In our survey, data on the characteristics of 80 centenarians in their younger days were obtained mainly from their families. It was found that they tended to be extroverted (81.2%), cheerful and friendly, and optimistic, while they were also hard workers and scrupulous.<sup>10)</sup> A national survey carried out later in 1975 also revealed similar features.<sup>11)</sup> However, it is unclear to what extent these character and personality traits are peculiar to centenarians.

### Quality of Centenarians' Lives

Our recent study of centenarians provided a better understanding of the quality of their lives.<sup>6)</sup> Many of them had a decreased current level of functioning. In fact, 22.2% of men and 41.1% of women fell under the category of "bedridden". This, however, is understandable: nearly one-half of the centenarian population is replaced every year, indicating that those in the terminal stage of life accounted for a considerable proportion at the time of investigation. However, it is apparent that they have led an independent life for most of their existence, with only a few people bedridden for a prolonged period. Therefore, they have a generally good view of their health status. As shown in Fig. 5, as many as 74.7% of men and 62.5% of women feel that they are in good health.

Those who had a hobby accounted for 46.6% of men and 26.9% of women, which seemed to be correlated with the degree of independent

Fig. 5 Centenarians self-rated health<sup>6)</sup>

functioning in life. A total of 43.6% of men and 25.8% of women found their lives worth living, whereas 28.4% of men and 33.6% of women did not; 27.9% of men and 40.6% of women were unclear. It is difficult to obtain accurate answers from centenarians with intellectual impairment.

Understandably, few centenarians participate in social activities. Only 20.5% of men and 11.6% of women are involved in activities such as work and volunteer service. However, such active centenarians accounted for a relatively high proportion among those who are functioning independently in life.

The presence of centenarians contributes to society through their embodiment of human longevity, their role as living witnesses to history, and their ability to transmit wisdom to the next generation. The 21st century is a time of mutual cooperation, in which the contribution of the elderly to society is indispensable, as is the support of the younger generation in caring for society's elders.<sup>12)</sup> The contribution made by the elderly to society is broad in scope, and the way centenarians contribute to society has great significance.

## REFERENCES

- 1) Shibata, H.: *Long Life, Peaceful Death – Live long happily and die happily*. Hokendojinsha Co., Ltd., Tokyo, 1994. (in Japanese)
- 2) Mazess, R.B. and Forman, S.H.: Longevity and age exaggeration in Vilcabamba, Ecuador.

*J Gerontol* 1979; 34: 94–98.

- 3) Shinkai, S.: Investigation of health and welfare in centenarians. *Report of the Study on Mental and Physical Health Promotion for Elderly People to Live a Free and Independent Life*. Japan Health Promotion & Fitness Foundation, 1999; pp.173–207. (in Japanese)
- 4) Shibata, H., Haga, H., Nagai, H. *et al.*: Nutrition for the Japanese elderly. *Nutr Health* 1992; 8: 165–175.
- 5) Uemura, M., Ogihara, R., Shibata, H. *et al.*: The effects of experiencing a fall on the activities of daily living (ADL) in Japanese centenarians. *Jpn J Prim Care* 2002; 25: 8–18.
- 6) Japan Health Promotion & Fitness Foundation: *Lives of Japanese Centenarians*; 2001. (in Japanese)
- 7) Watanabe, S. and Shibata, H.: Centenarians in Japan: Geographic distribution of centenarians. *Geriatr Med* 2000; 38: 1269–1276. (in Japanese)
- 8) Japan Health Promotion & Fitness Foundation: *1981 Report of the Health and Nutrition Survey on Long-lived People*; 1982. (in Japanese)
- 9) Japan Health Promotion & Fitness Foundation: *1993 Report of the Survey on Health and Welfare of Long-lived People*; 1995. (in Japanese)
- 10) Tokyo Metropolitan Institute of Gerontology: *Report of the Survey on Centenarians*; 1975. (in Japanese)
- 11) Elderly People's Welfare Development Center: *Integrated Study Report of Long-lived People*; 1976. (in Japanese)
- 12) Shibata, H.: *More than 80% of the Elderly Are Independent!* Business-Sha, Tokyo, 2002. (in Japanese)

# Forefront of Treatment for Abdominal Aortic Aneurysm

JMAJ 47(7): 344–349, 2004

Hiroshi YASUHARA

*Professor, Teikyo University School of Medicine, Ichihara Hospital*

**Abstract:** This paper is a comprehensive review of the latest treatments for abdominal aortic aneurysm, with primary reference to endovascular stent graft placement. While there has already been an established operative procedure for the treatment of abdominal aortic aneurysms, i.e. surgical excision of aortic aneurysm upon laparotomy and vascular replacement with prostheses, further developments are under way toward minimally invasive approaches with emphasis on quality of life of patients. Most noteworthy is the advent of endovascular stent graft placement, which has been introduced into the clinical setting; there are already reports of the short-term results of the procedure. On the other hand, new problems peculiar to endovascular stent graft placement have been emerging, leading to various consequences. The problems may be roughly categorized as: 1) problems related to surgical techniques, 2) problems involving the surgeons conducting the treatment, and 3) medicoeconomical problems. These problems should be resolved by further assessments from the standpoint of patient primacy.

**Key words:** Abdominal aortic aneurysm; Endovascular stent graft placement; Minimally invasive surgery

## Introduction

The history of surgical operation in the treatment of abdominal aortic aneurysm began with the attempt made by Charles Dubost *et al.*<sup>1)</sup> involving vascular allografting upon resection of an aortic aneurysm. Through the subsequent improvement of operative techniques and medical materials over the past five decades or so,

the vascular replacement with prostheses currently being undertaken has become established as a standard procedure. Materials used for vascular prostheses are usually of two types, i.e., the nonwoven fiber ePTFE and the knitted synthetic Dacron. Dacron tubes sealed with such readily decomposed and absorbable substances as fibrin, albumin, or gelatin in order to prevent blood leakage have come into fre-

---

This article is a revised English version of a paper originally published in the *Journal of the Japan Medical Association* (Vol. 128, No. 11, 2002, pages 1651–1655). The Japanese text is a transcript of a lecture originally aired on June 6, 2002, by the Nihon Shortwave Broadcasting Co., Ltd., in its regular program “Special Course in Medicine”.

quent use recently. Therefore, the need for the conventional process of preclotting has been diminishing.

The current surgical procedure for treating abdominal aortic aneurysms may well be said to be almost completed, although the medical materials being used are still being improved little by little. The high degree of perfection of the procedure is reflected in the low postoperative mortality rate; reports documenting postoperative mortality rates of  $\leq 1\%$  have become standard in recent years, compared to the previous 5 to nearly 10%. Surgery-related deaths are reportedly nil at some institutions.

Nevertheless, this apparently perfected operative procedure has recently been undergoing innovative changes through endovascular stent grafting incorporating endovascular surgical techniques. This article will briefly review the endovascular stent grafting process, and consider changes currently occurring at the forefront of treatment for abdominal aortic aneurysms and the problems inherent therein.

### Stent Graft Endovascular Placement for Repair of Abdominal Aortic Aneurysm and Its Characteristic Features

Endovascular surgery originates from the percutaneous transluminal angioplasty (PTA) initiated by Dotter and Jodkins<sup>2)</sup> and is aimed at treatment of vascular lesions with minimal operative invasion, mainly using catheters. The major distinctive feature of stent graft placement with the application of the techniques of endovascular surgery, compared to the conventional surgery for abdominal aortic aneurysm, consists in its minimal invasiveness, which places a great deal less pain and stress on the patient. The surgical wound involved in stent grafting measures only a few centimeters extending to expose the unilateral or bilateral femoral artery or iliac artery; whereas in the conventional operative procedure, the wound extends much farther, from just below the xiphoid process down to just above the superior margin of the

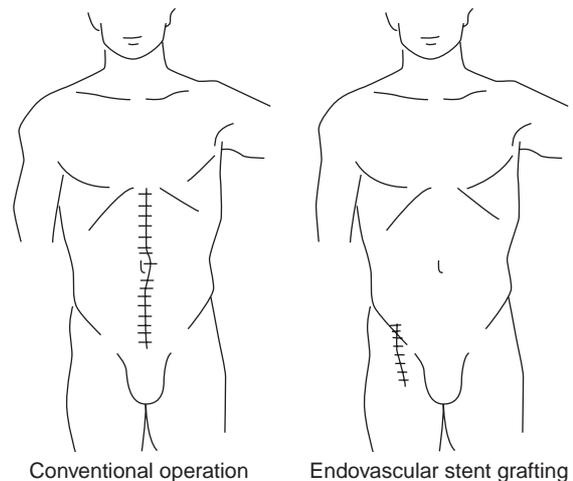


Fig. 1 Comparison of surgical wounds

pubes (Fig. 1).

A folded prosthetic graft is led, as it stands, into the lumen of an abdominal aortic aneurysm through the exposed femoral artery or iliac artery. This article will not pertain to individual types of stent grafts. The artificial graft is made of thinner knitted Dacron or ePTFE so that it can be folded into a fine compact form, and is reinforced with a stent to endow the vessel with strength when unfolded. The graft led into the lumen of an abdominal aortic aneurysm is deployed in an undistended aortic region proximal to the aneurysm, and is fixed fast with the stents to the sites of origin and terminus of the aortic aneurysm. Thereby, the stent graft is implanted inside of the aneurysm (Fig. 2).

Formerly, stent grafts were available only in straight form and, therefore, surgical excision with grafting was indicated only for repair of aortic aneurysms localized in the abdominal aorta without involving distention of the iliac artery. Recently, however, abdominal aortic aneurysms with associated iliac artery distention have also become subject to the treatment thanks to methods for combining two stent grafts. A variety of systems of graft anchorage with stents have been devised, with two different endoluminal fixation systems in current use,

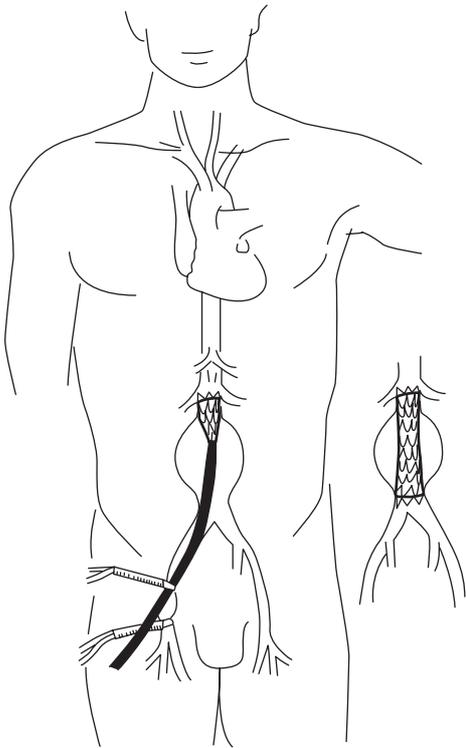


Fig. 2 Endovascular stent grafting

i.e. the anchoring of the stent onto the arterial wall by means of inflating a balloon from inside the stent, and that by the self-expansive force of the stent spring *per se*. Thus, stent grafting is aimed at treatment of aortic aneurysm by intercepting blood flow in the space between the external surface of a prosthetic vessel wall and the inner surface of the wall of the abdominal aortic aneurysm, and by forming thrombosis within the blood flow interception space (Fig. 2).

Although the physical burden upon the patient can be substantially reduced, the degree of invasiveness into the vasculature itself does not much differ from that of other procedures, since artificial graft and stents are used in the procedure of endoluminal stent grafting. As mentioned above, the operation of endoluminal stent grafting requires no laparotomy, whilst a laparotomy entailing a large surgical wound has to be carried out in usual surgical treatment of abdominal aortic aneurysms. The postoperative clinical course differs greatly between the

Table 1 Problems Inherent in Endovascular Stent Grafting

- |  |
|--|
| 1) Problems related to the operative technique <i>per se</i> |
| 2) Problems related to the doctor conducting the operation   |
| 3) Problems related to medicoeconomics                       |

procedures. Reports have documented an earlier start to oral dietary intake after the operation, and earlier leaving of the sickbed and hospital discharge owing to the lower severity of the surgical wound in patients treated with endoluminal stent grafting, as compared with those treated by conventional operative techniques. Consequently, the duration of the patient's hospital stay is significantly reduced.

### Points at Issue Inherent in Endoluminal Stent Grafting

From what has been described herein, one might have the impression that endoluminal stent grafting will quite naturally supplant the conventional surgery, which is highly invasive. Unfortunately, however, several problems have been pointed out regarding this surgical approach, and may well hinder the spread of the transluminal procedure.

The points at issue in endoluminal stent grafting are summarized in Table 1. They include 1) problems related to the operative technique *per se*, 2) those related to the doctor conducting the operation, and 3) those related to medicoeconomics. Needless to say, these problems are in no way independent, but are mutually and closely interrelated. However, summarization in this fashion may facilitate the readers' understanding of the problems in the practice of endoluminal stent grafting.

#### 1. Problems related to the operative technique *per se*

Let me refer first to problems related to the

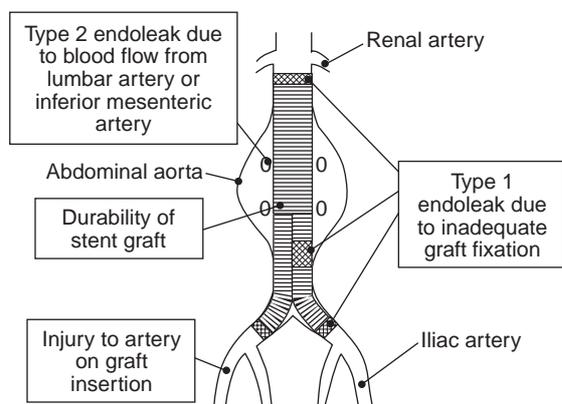


Fig. 3 Problems in endovascular stent grafting for abdominal aortic aneurysm

operative technique *per se*. In the conventional standard procedure of replacement of an aortic aneurysm with a synthetic graft, as mentioned above, the aortic blood flow is intercepted and then the aortic aneurysmal wall is incised, followed by placing sutures on the lumbar artery for hemostasis. No such hemostasis by suturing is required in the endoluminal stent grafting; hence, in some cases, blood flow from the lumbar artery remains in the space surrounded by the stent graft and the wall of the abdominal aortic aneurysm where the blood flow should be intercepted as intended.

Such blood flow persistence may occur due to other causes as well. Aneurysms of the abdominal aorta arise in most cases in the segment inferior to the takeoff of the renal arteries and, as the site of aneurysmal origin may be distal to that of the takeoff, the proximal end of the inserted graft is fixed with the stent onto the undistended site of the aorta. In a case where the distance is too short, however, the graft may cause occlusion of the renal artery, or fixation of the stent may become unstable, resulting in persistent blood flow into the aneurysm by the side of the stent. Such blood flow persistence within the aneurysm is termed “endoleak.” It is classified into two types: “type 1 endoleak”, which is attributable to the stent graft itself, and “type 2 endoleak”, which

is ascribed to collateral blood flow such as that from the lumbar artery.

Recently, the fixation system for the proximal end of the stent graft has been improved so that the stent may even approach the takeoff of the renal arteries,<sup>3</sup> yet the problem of “endoleak” has not been fully resolved.<sup>4</sup> Moreover, it is known that type 1 blood leakage may also occur from the peripheral fixation site or from the graft junction site of a combined Y-shaped stent graft, besides blood flow persistence from the proximal end of the stent graft (Fig.3).

The various problems described here pertain particularly to operative techniques and early postoperative complications. Due to the relatively short history of endoluminal stent grafting (which covers about a decade), long-term results are yet to be scrutinized. In fact, some cases have been reported in which a stent graft placed in a markedly tortuous abdominal aortic aneurysm caused a gradual further advance in tortuosity, eventuating in aortic perforation by the stent; and other cases have been reported where an aneurysm continued to expand due to “endoleak” postoperatively, resulting in rupture of the aneurysm.<sup>5,6</sup> It is considered that the durability of the synthetic vascular graft must be fully assessed in the future, because a thin graft material is utilized so as to facilitate insertion of a stent graft through small arteries.<sup>6</sup>

## 2. Problems related to the doctor conducting the treatment

Now I would like to discuss the problems related to the doctors’ specialty conducting endovascular stent grafting. The status quo is that the treatment is undertaken by cardiovascular surgeons or medical radiologists with their own respective procedures, without active mutual intercommunication. Endovascular stent grafting for treatment of abdominal aortic aneurysm is likewise performed by vascular surgeons as well as by medical radiologists in the United States, where there is an advanced medical specialty board system. Even in the United States, however, surgeons formerly regarded it

as heretical that this treatment was not performed by one of themselves. Among radiologists, on the other hand, this treatment was generally recognized as being performed by radiologists, since they had gained substantial results with treatments involving catheterization as an interventional radiological approach. With the improvement in therapeutic outcomes with endovascular stent grafting achieved during the last several years, however, the recognition that this treatment should be carried out by vascular surgeons is increasingly being accepted among vascular surgeons, as they have long-standing experience in operations on abdominal aortic aneurysms. Recently, cardiovascular medical interventionalists experienced in PTCA and stenting of the coronary artery have also been participating in this realm. The situation as to what type of professional training is required for a physician to conduct this treatment is now chaotic.

Such steering of patients from the viewpoint of the physician, however, is not by any means favorable to the patient. Many surgeons feel that the procedure should be performed by physicians adequately trained in surgery, just in the case of laparoscopic cholecystectomy, which came to be performed by surgeons versed in the knowledge and techniques of laparotomic cholecystectomy. From an entirely different viewpoint, however, there are surgeons who point out the possible advent of endovascular surgeons distinct from the conventional concept of the cardiovascular surgeon. Solution of this problem regarding the training required for this new treatment modality should thus be sought urgently after candid discussions.

### **3. Problems related to medicoeconomics**

Finally, I would like to discuss the problems related to medicoeconomics as a subject implicit in endovascular stent grafting.

In this therapeutic approach, medical materials that have not yet been standardized are used as mentioned above. The fact that a wide variety of medical materials are used does in

itself directly indicate a low degree of perfection of the treatment, but what we ought to take note of here is that there are also other factors contributory to the use of various medical materials in this treatment. In other words, the stent graft to be used in the present endovascular stent grafting must be individualized/tailored for a given patient, so that the characteristics and disease state of the patient, such as the build, size and tortuosity of the aorta, patency of the lumbar artery, and so forth largely influence the therapeutic outcome. Endovascular stent grafting is basically a tailor-made therapy, and consequently the medical materials used and the medical fees involved are highly expensive.

With endovascular stent grafting, a significant reduction in the duration of the hospital stay can be anticipated as compared to laparotomic corrective procedures, and this may help to reduce the treatment fee. It is difficult, however, to estimate the medico-economical implications of endovascular stent grafting, because the procedure involves the use of expensive, cumbersome medical materials. Although it is unquestioned that the cost of medical materials will gradually decrease with the future spread of this treatment method, it remains to be clarified to what extent the cost will be reduced.

### **Other New Treatments for Abdominal Aortic Aneurysm**

I have reviewed endovascular stent grafting as a therapy for abdominal aortic aneurysm, and considered the problems inherent in the therapy. Now, from a different angle, we would like to briefly review other attempts towards surgical treatment for abdominal aortic aneurysms.

In the field of surgery, attempts at minimally invasive surgical interventions utilizing laparoscopy or robots have been actively undertaken in recent years. These attempts are designed to perform operative manipulations beyond

human capability, with the aid of surgical instruments to minimize surgical invasiveness. Laparoscopes and robots are capable of entering an incised wound which is so fine and small that human fingers cannot pass through it, and enable operations within the body. Operations such as endoscopic gastric mucosectomy are considered an ultimate target, and can be conducted without leaving any wound on the body surface.

In the surgery for abdominal aortic aneurysms, as well, attempts at minimal incision surgery to carry out conventional vascular replacement with a synthetic prosthesis through a smaller surgical wound are being made with the improvement of the retractor or with the application of laparoscopic surgical instruments. Although intra-abdominal operations remain the same as with conventional procedures and may therefore differ little from the latter in surgical invasiveness in the strict sense of the term, it seems highly likely that the physical burden on the patient may be substantially reduced by minimizing laparotomic manipulations, as in the case of laparoscopic cholecystectomy. For those surgeons skilled in conventional operative procedures, furthermore, the treatment is considered to have the advantage of being more acceptable.

## Conclusion

I have reviewed surgical treatments such as endovascular surgery with stent grafting in their roles at the forefront of therapy for abdominal aortic aneurysms. Endovascular surgery with stent grafting has been dramatically

altering the conventional surgical treatment, and the treatment for abdominal aortic aneurysms is considered to be continuing to progress. It is also anticipated that, in the future, abdominal aortic aneurysms be prevented by medicinal and dietary therapy upon elucidation of the pathogenetic mechanisms of atherosclerosis based on analysis of genetic information, rather than by placing emphasis on treatments involving surgical intervention.

## REFERENCES

- 1) Dubost, C., Allary, M. and Oeconomos, N.: Resection of an aneurysm of the abdominal aorta. *Arch Surg* 1952; 64: 405–408.
- 2) Dotter, C.T. and Jenkins, M.P.: Transluminal treatment of atherosclerotic obstruction: Description of a new technique and preliminary report of this application. *Circulation* 1969; 30: 645–670.
- 3) Bove, P.G., Long, G.W., Zelenock, G.B. *et al.*: Transrenal fixation of aortic stent-grafts for the treatment of infrarenal aortic aneurysmal disease. *J Vasc Surg* 2000; 32(4): 697–703.
- 4) Wisselink, W., Cuesta, M.A. and Berends, F.J. *et al.*: Retroperitoneal endoscopic ligation of lumbar and inferior mesenteric arteries as a treatment of persistent endoleak after endoluminal aortic aneurysm repair. *J Vasc Surg* 2000; 31(6): 1240–1244.
- 5) Guidoin, R., Marois, Y., Douville, Y. *et al.*: First-generation aortic endografts: analysis of explanted Stentor devices from the EUROSTAR Registry. *J Endovasc Ther* 2000; 7(2): 105–122.
- 6) Beebe, H.G., Kritpracha, B., Serres, S. *et al.*: Endograft planning without preoperative arteriography: a clinical feasibility study. *J Endovasc Ther* 2000; 7(1): 8–15.