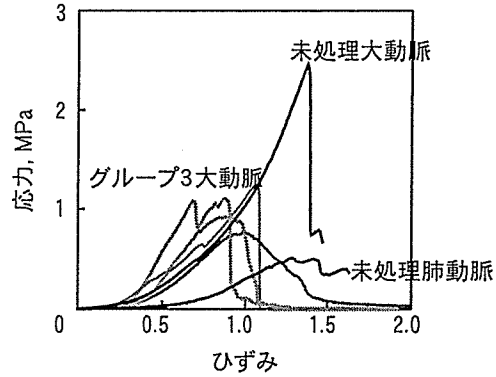

図４　グループ３の下行大動脈における力学特性

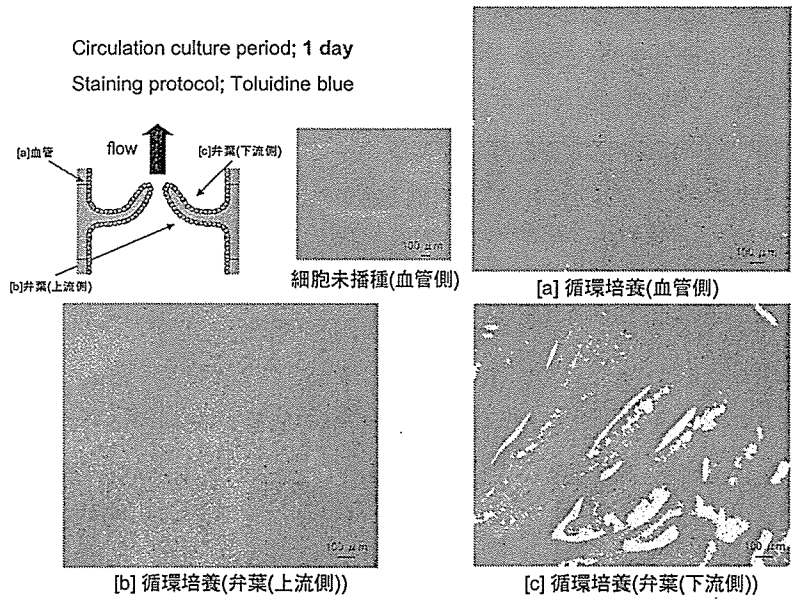

図５　脱細胞化大動脈弁への血管内皮細胞の播種

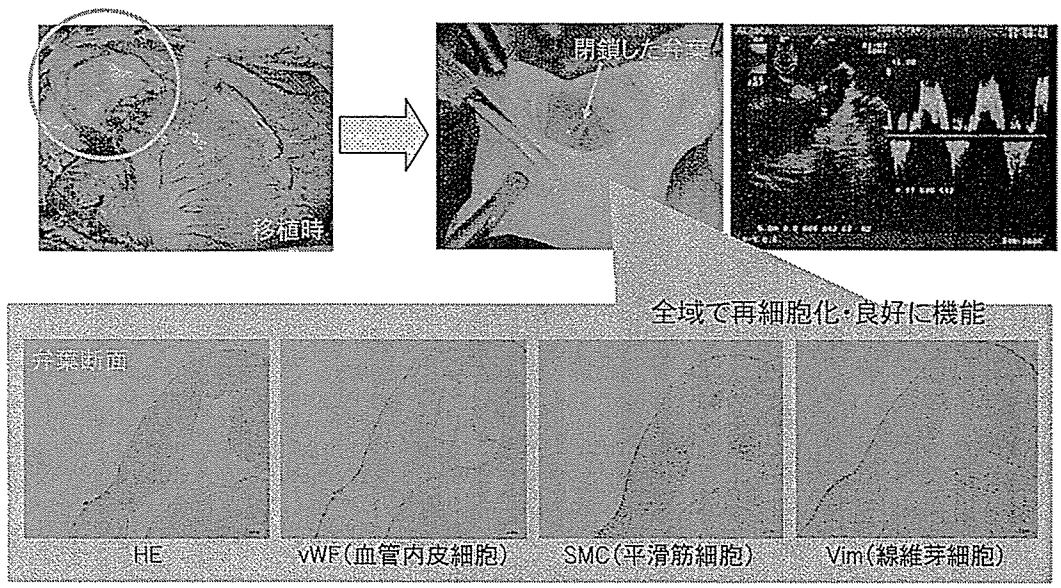

図６　グループ１の肺動脈弁における移植６ヶ月後所見

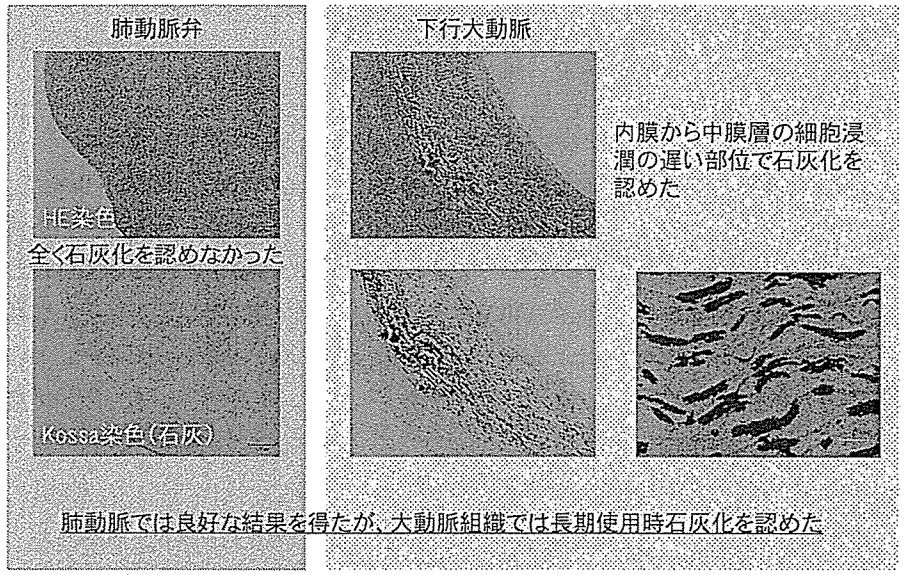

図７　グループ１の移植６ヶ月後の石灰化所見

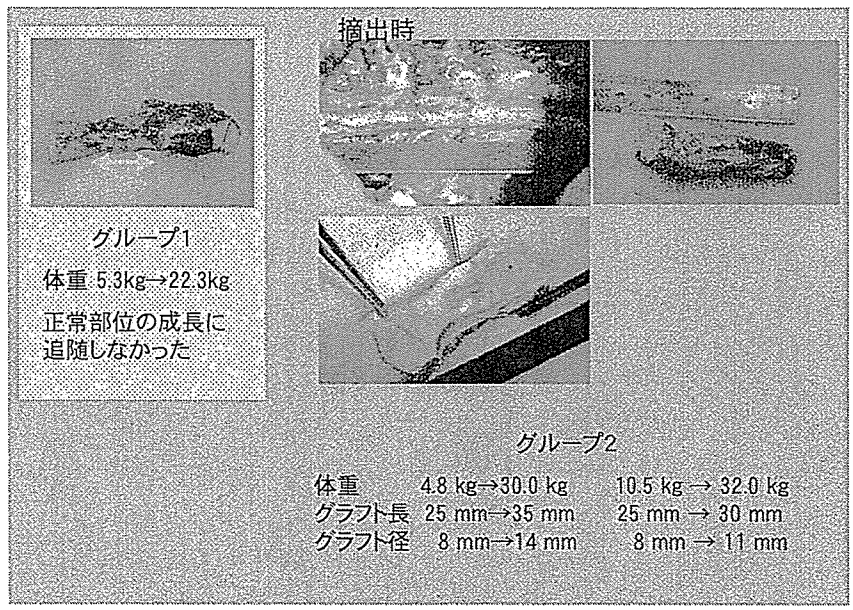

図８　下行大動脈の移植12ヶ月後所見

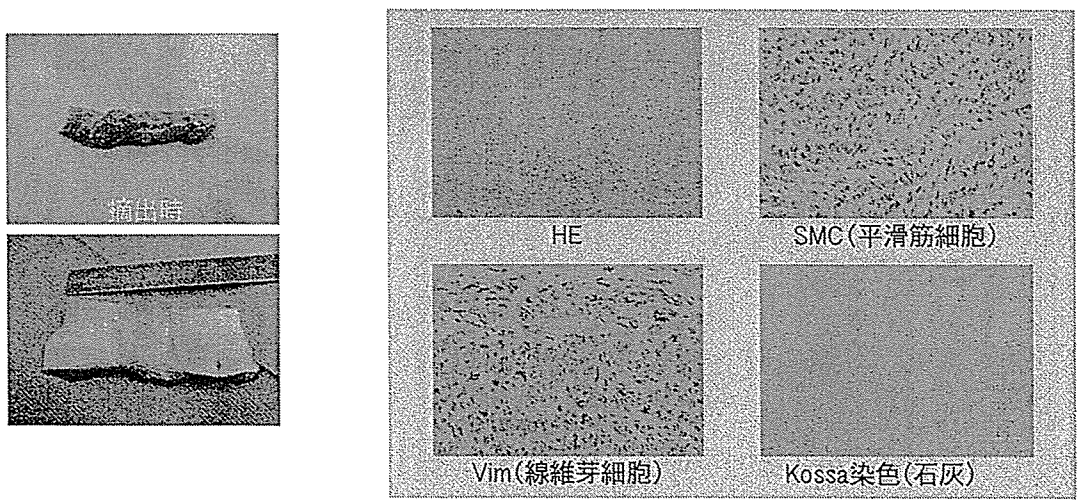

図９　グループ３の下行大動脈における移植３ヶ月後所見

表１．細菌培養検査結果

	Streptocossus constellatus	
	fragment sample	homogenized sample
Native tissue	(+)	2+
Decellularized tissue	not isolated	not isolated

別添４

研究成果の刊行に関する一覧表

書籍

	著者氏名	論文タイトル	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
1	Fujisato T, Minatoya K, Yamazaki S, Yin M, Niwaya K, Kishida A, Nakatani T, Kitamura S	Preparation and recellularization of tissue engineered bioscaffold for heart valve replacement	Mori H, Matsuda H	Cardiovascular regeneration therapies using tissue engineering approaches	Springer-Verlag	Tokyo	2004	83-94
2	岸田晶夫	生体適合性評価法	樋口亜紺	医療用マテリアルと機能膜	シーエムシー出版	東京	2005	51-60
3	岸田晶夫	人工心臓膜	樋口亜紺	医療用マテリアルと機能膜	シーエムシー出版	東京	2005	82-8
4	山岡哲二、木村良晴、藤里俊哉	医療用バイオベースマテリアル	木村良晴、小原仁実	バイオベースマテリアルの新展開	シーエムシー出版	東京	2007	279 (187-97)
5	藤里俊哉、北村惣一郎	心臓弁	筏　義人	再生医療工学の技術	シーエムシー出版	東京	2007	251 (142-7)

雑誌

	発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
1	Kimura T, Okuno A, Miyazaki K, Furuzono T, Ohya Y, Ouchi T, Mutsuo S, Yoshizawa H, Kitamura Y, Fujisato T, Kishida A	Novel PVA-DNA nanoparticle prepared by ultra high pressure technology for gene delivery	Mater Sci Eng C	24	797-801	2004
2	Numata S, Fujisato T, Niwaya K, Ishibashi-Ueda H, Nakatani T, Kitamura S	Immunological and histological evaluation of decellularized allograft in a pig model: Comparison with cryopreserved allograft	J Heart Valve Dis	13 (5)	984-90	2004
3	Fukuhara S, Tomita S Nakatani T Fujisato T, Ohtsu Y Ishida M, Yutani C Kitamura S	Bone marrow cell-seeded biodegradable polymeric scaffold enhances angiogenesis and improves function of the infarcted heart	Circ J	69 (9)	850-7	2005
4	岸田晶夫、藤里俊哉	再生医療用材料	日本再生医療学会誌	4 (4)	546-53	2005
5	菅　理晴、藤里俊哉 永谷憲歳、中谷武嗣	ブタ組織の脱細胞化	移植	40 (5)	441-4	2005
6	岸田晶夫	生体材料の遺伝子発現による評価	材料の化学と工学	42 (4)	18-22	2005
7	岸田晶夫	再生医療のための脱細胞化生物組織（バイオスキャフォールド）	生体材料工学研究所年報	39	9-12	2005
8	中谷武嗣、富田伸司 永谷憲歳.	重症心不全に対する幹細胞による心筋再生療法の開発	再生医療	4	399-403	2005
9	中谷武嗣、川西秀樹	ハイブリッド人工臓器の現状と未来　第42回日本人工臓器学会大会座長報告	人工臓器	34	39-40	2005
10	澤田和也、寺田堂彦、藤里俊哉	繊維と線維（生体繊維の洗浄と再生医療への展開）	繊維と工業	63 (5)	120-4	2007
11	Fujisato T, Niwaya K, Minatoya K, Kishida A, Nakatani T, Kitamura S	Reduction of Antigenicity and Risk of Infection in Regenerative Tissue Transplantation by Cold Isostatic Pressing	High Pressure Bioscience and Biotechnology	1 (1)	161-5	2007
12	Kimura T, Iwai S, Moritan T, Nam K, Mutsuo S, Yoshizawa H, Okada M, Furuzono T, Fujisato T, Kishida A	Preparation of poly(vinyl alcohol)/DNA hydrogels via hydrogen bonds formed on ultra-high pressurization and controlled release of DNA from the hydrogels for gene delivery	J Artif Organs	10 (2)	104-8	2007

Preparation and Recellularization of Tissue Engineered Bioscaffold for Heart Valve Replacement

Toshia Fujisato[1], Kenji Minatoya[2], Sachiko Yamazaki[2], Yin Meng[4], Kazuo Niwaya[2], Akio Kishida[3], Takeshi Nakatani[4], and Soichiro Kitamura[2]

[1]Department of Regenerative Medicine & Tissue Engineering, [2]Cardiovascular Surgery, [3]Biomedical Engineering, and [4]Organ Transplantation, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

Summary. Tissue engineered grafts based on polymeric or acellular xenogeneric matrices have been widely studied, and found to have greater durability and functionality with growth potential and less immunogenicity than current bioprostheses. On the other hand, there are still several problems to be solved such as degradation control of biodegradable polymeric scaffolds and unwanted transfer of unknown animal related infectious diseases. In this chapter, our novel tissue processing of decellularization named PowerGraft by ultrahigh pressure treatment for safe tissue transplantation is reported. Porcine heart valves were isolated under sterile conditions and treated by cold isostatic pressing (CIP) at 4°C for disruption of donor cells. The cell debris was then washed out in PBS under microwave irradiation at 4°C. The tissues were completely cell free when they were treated by a CIP of 980 MPa (10,000 atm) for 10 min. There was no porcine endogeneous retrovirus (PERV) detected in the treated tissue. There were no significant changes in biomechanical properties of breaking strength and elastic modulus. From the in vitro incubation test, the tissues were disinfected when CIP was applied to the tissues contaminated by normal bacteria floras. The endothelial cells were well seeded on the acellular bioscaffold by the roller and circulation culture systems sequentially. This PowerGraft processing may provide a more durable and safe bioscaffold for tissue transplantation.

Key words. Scaffold, Acellular tissue, PERV, High pressure, Microwave

Introduction

The artificial heart valve is one of the most clinically used artificial devices applied to about 300,000 patients per year worldwide, whereas it has still several shortcomings should be solved. The mechanical valve made of the pyrolytic carbon has good durability that might be longer than the patient's life time, however it has poor biocompatibility due to blood coagulation and patients must take an anti-coagulant drug under strict regulations throughout the rest of their lives. This drug, warfarin, is teratogenic and the female patient who wants to have a baby can not receive a mechanical valve. The xenograft valve made of the chemically crosslinked porcine valve or bovine pericardium in order to minimize the host's immune reaction has good biocompatibility, and hemodynamics, and is resistant to infections. The use of the xenograft valve is on the increase since it is superior to the mechanical valve in the quality of life, because it does not require any administration of an anti-coagulant drug. However, the durability of the xenograft is shorter than the mechanical valve, being about 15 to 20 years in elderly and 5 to 10 years in younger patients, due to calcification of the glutaraldehyde-fixed animal tissue. It is recommended that the xenograft should be used for the elderly patient over 65 years old in the guidelines of the American Heart Association and the American Association of Thoracic Surgeons.

Thanks to the establishment of tissue banks in this decade, some patients have had their defective tissues (heart valve, blood vessel, skin, and bone) replaced with cryopreserved donated tissue from a cadaver, rather than the current imperfect artificial devices.

The cryopreserved allograft valve, referred as the homograft valve, is clinically available in many countries and has been reported to have good clinical results. The homograft valve has the advantages of better biocompatibility compared to the mechanical valve, in durability to the xenograft valve, and in resistance to infections the both valves. However, the limitation on homograft valve availability might never be improved even in the

future. The Ross operation, in which the dysfunctional aortic valve is replaced by the patient's own autologous pulmonary valve and the homograft valve is implanted at the compromised pulmonary position, has been reported to have good clinical results especially in pediatric patients. The autologous tissue does not evoke an immune rejection and becomes bigger in size depending on the patient's growth. Since the other mechanical, xenograft, and homograft valve remains as an exogenous material in the patient's body and never grows, the pediatric recipients must have multiple operations through their lives.

To overcome these shortcomings in the current mechanical and biological heart valves, many research groups have been developing tissue engineered (TE) heart valves with properties similar to autologous valve tissue. Since the TE valves might be substituted by the host cells and tissues after the transplantation, the recipients can enjoy their good biocompatibility, durability, and growth potential.

TE heart valve

For the recovery of defected tissues, substitutional scaffolds must be implanted for tissue regeneration. There are two approaches that allow the scaffold materials to realize the TE tissues. One approach is using artificial biodegradable polymeric materials such as polylactic acid, polyglycolic acid, and polycaprolacton. Prof. Shin-oka and his group have reported successful clinical experiences of about 50 patients implanted with TE blood vessels made of the polyglycolic acid seeded with the patients' autologous bone marrow cells (see the chapter by Shin-oka T, this volume). However, the biodegradable polymeric materials are generally stiffer than the native biological tissues and do not easily take the same shape and structure as the biological tissues. Especially for aortic heart valve replacement, the scaffold requires flexible mechanical properties and strict degradation control for sufficient strength against the blood pressure.

The other approach is using acellular tissues for the scaffold as described in this chapter in which the cells and antigen molecules are removed to diminish the host tissue reaction. The acellular scaffold may have the same structure and composition as the natural tissue and be regu-

lated by interaction with the host tissue cells. CryoLife, Inc. (Kennesaw, GA) is the first company that provided acellular heart valves and blood vessels both from allogeneic and xenogeneic tissues. This company obtained a patent for the decellularization process using the gentle enzymatic treatment named SynerGraft® technology in 1994 and put the decellularized porcine heart valves (SynerGraft® Heart Valve, Model 700) on the market of Europe in 2001. It was reported in 2001 that they were successfully repopulated in a few months after the transplantation (Elkins et al. 2001). Whereas the multicenter clinical outcomes in Europe and Australia for the reconstruction of the right ventricular outflow tract in pediatric patients from 2001 to 2002 showed that only 7 of 19 valves remained implanted and clinically functional at the last follow-up, with 4 deaths (Simon et al. 2003 and R. Chard et al. 2004). On the other hand, the multicenter registry of the decellularized allograft both of pulmonary and aortic valves (CryoValve® SG) in USA from 2000 to 2003 demonstrated excellent clinical performance with more than 92% patient survival after 2 years transplantation (Clarke et al. 2004). Factors on the failure in the xenogeneic decellularized valves were not clear but were presumed to be mainly the result of the processing methods and remaining xenogeneic cell debris inside the tissue. Prof. Konertz and his group in Germany have also started clinical trials of the porcine pulmonary heart valves decellularized by the sodium-deoxycholate named AutoTissue technology in Ross procedure since 2002 (Dohmen PM, et al. 2002). They have reported that the pulmonary grafts named Matrix P showed excellent postoperative results with only 1 death in more than 120 patients and no functional failure and calcification in the grafts (Konertz WF. 2004). Prof. Haverich and his group in Germany have started clinical study too on decellularized allograft valves seeded by the patients' endothelial progenitor cells from 2002 and reported successful results (Teebken OE, et al. 2003 and S. Cebotari et al. 2004). They have been using the detergent Triton® X-100 (Bader A, et al. 1998) or the enzyme trypsin as the agent for the decellularization. In addition, there are several research groups developing acellular heart valves such as Prof. Ingham (Booth C, et al. 2002) in England and Prof. Stock (Schenke-Layland K, et al. 2003) in Germany.

We have been developing acelluar scaffolds for heart valve, blood vessel and trachea made of porcine tissue and their patients' autologous recellularization in vitro for the custom-made tissue transplantation since 2000 (Fig. 1). The scaffold with autologous cells may be replaced by the host tissue by the remodeling process regulated by the surrounding cells through digestion of the scaffold matrices and production of the autologous extracellular matrices. After the remodeling had been completed, the implanted tissue may be identical with an original autologous tissue and may have growth potential. Also, the recellularized grafts may enhance the functional performance such as anti-coagulation and anti-calcification in the early stage of the postoperation.

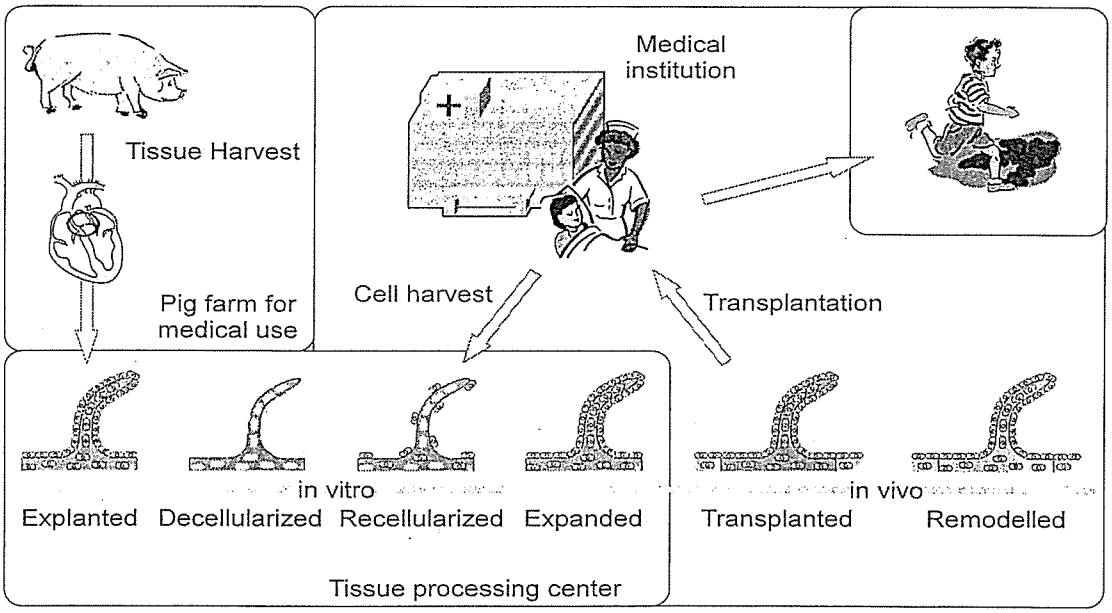

Fig. 1. Custom-made tissue transplantation.

Power Graft technology

All of the above groups are using detergents and/or enzymes as decellularization media such as Triton® X-100, sodium dodecyl sulfate, deoxycholate, trypsine, DNase, and RNase. We have started to investigate decellularization of porcine heart valves using Triton® X-100 and found that the cells in the cusps were unstained by H-E staining after 6 hrs treatment,

however cells still stained deep inside the basal tissue of cusps even after 24 hrs incubation regardless of the detergent concentrations of 0.5%, 1% and 2.5% (Fig.2). Under scanning electron microscopy, gaps between endothelial cells were observed after more than 3 hrs of treatment, however residues of the endothelial cells on the basal membrane were still attached. Since the detergents are generally cytotoxic and it takes time for their removal before the transplantation and cell seeding, it may lead to denature of biological properties and contamination in the process. Recent BSE (Bovine Spongiform Encephalopathy) and vCJD (variant Creutzfeldt-Jakob disease) issues have been affecting tissue transplantation from the point of view of safety. Especially if the scaffolds are prepared from animal tissues, the animal cell components must be removed completely for the prevention of unknown transfer of animal related infectious diseases. In addition, if the tissue source is porcine, the removal of porcine endogenous retroviruses (PERVs) that have ability of infection to the human cells in vitro must be validated (Magre S, et al. 2003). However, it is not easy to remove the cell components completely in the decellularization process by the detergent and proteinase as described above because of the limited permeability of the agents.

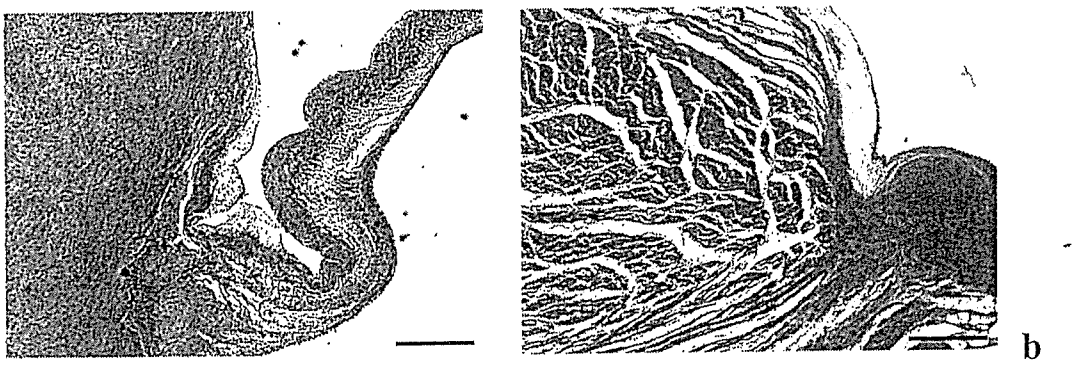

Fig. 2a. b. Decellularization of porcine heart valve basal tissue by detergent. **a** Native. **b** Treated by 1% Triton® X-100 for 24 hrs. Scale bars are 500 μm.

We have introduced a novel decellularization process to create a safe tissue scaffold by the combination of ultrahigh pressure treatment of the cold isostatic pressing (CIP) and washing under the microwave irradiation named PowerGraft technology. During CIP, when fluid pressure is added to the material enclosed in a pressure vessel, the whole surface of the ma-

terial will receive the pressure evenly which is equal to the fluid pressure and then compressed without flattening. This CIP technology has already been established in the food industry and Meijiya Food Co., LTD, Japanese Jam factory, has already commercialized the world first food processed by the CIP in 1990. It has been reported that the functional proteins are denatured by pressing at about 300 MPa and the most of the viruses like Human Immunodeficiency Virus are inactivated at more than 600 MPa (Hayashi R. 2002).

The porcine aortic and pulmonary valves, aorta, and trachea were isolated from 6 month-old Clawn miniature pigs (Japan Farm Co. Ltd, Kagoshima, Japan) weighing about 15 kg under sterile conditions. The harvested tissues were washed and packed in sterile bags filled with PBS. The packed tissues were treated by ultrahigh pressure at 4 °C using a CIP apparatus (Kobe steel LTD, Kobe, Japan). They were then washed by PBS under microwave irradiation at 4 °C (Azumaya Medical Devices Inc., Tokyo, Japan) for accelerated removal of the residues of the broken cells from the CIP treated tissues. H-E staining of the cusps of porcine aortic heart valve showed that the tissues were completely cell free when the CIP of 970 MPa was applied for 10 min and washed under microwave irradiation for 5 days. The pulmonary valve, aortic tissue, and trachea were also completely cell free even in the cartilage tissue of the trachea (Fig.3). We have chosen the Clawn miniature pig as a donor animal since its size adapts to human tissues well and its genome has been well studied in order to develop a human gene induced transgenic animal for organ transplantation. There was no PERV products detected in a PCR assay from the aortic and tracheal tissues processed by the CIP, whereas it was still detected in the tissue treated by Triton® X-100 after 24-hr incubation (Fig. 4). Tissues pre-contaminated by the normal bacteria floras were decontaminated when treated at more than 485 MPa. There were no significant changes in biomechanical properties in terms of the breaking strength and elastic modulus of the leaflets treated at 970 MPa for 10 min. This was supported by elastica-van Gieson staining, which showed collagen and elastin fibers were well maintained in the bioscaffold tissue decellularized by the CIP. The effect of microwave irradiation is the same as the appliance of conventional microwave oven using the vibration of water molecules at 2,450

MHz. The principle of accelerating the washing time by microwave irradiation is still unclear, but it is presumed that the high-speed motion of water molecules and enhances the permeation of the tissues. It is not necessary to use the microwave for washing after the CIP treatment, however it makes washing time about one tenth of that compared to conventional incubation.

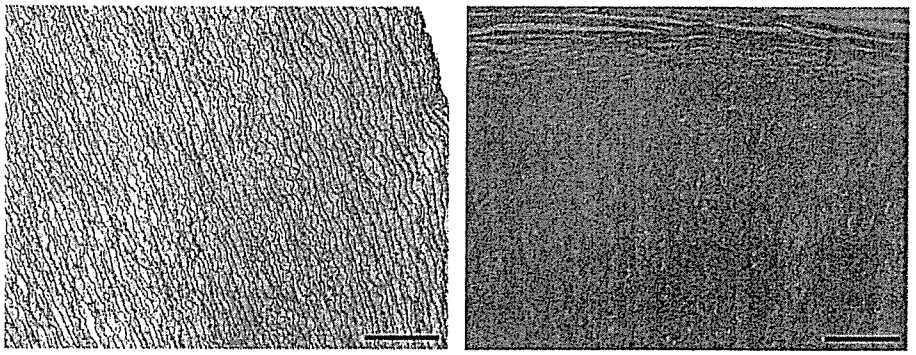

Fig. 3a. b. Decellularization by Power Graft technology using CIP at 980 MPa for 10 min. **a** Aorta. **b** Trachea. Scale bars are 100 μm.

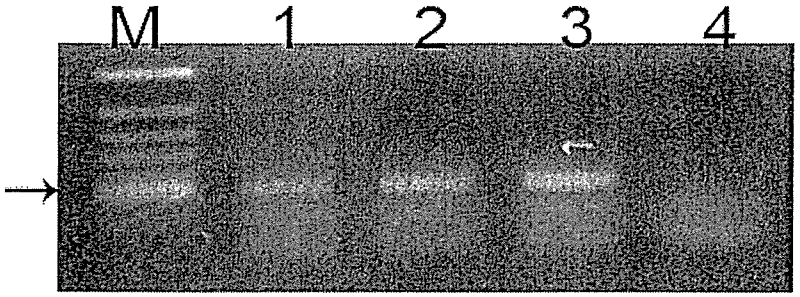

Fig. 4. PERV assay on the decellularized heart valves. (M: Marker, 1: Native, 2,3: 1% Triton® X-100 for 24 hrs, 4: PowerGraft of 980 MPa for 10 min)

Recellularization of Power Graft in vitro

The native heart valve tissue is fully covered with endothelial cells and is occupied mainly by smooth muscle cells. As described above, the incorporation of autologous cells to the acellular scaffold may maintain physiological activity and prevent calcification at the graft site during the early

stage. Endothelial cell seeding on the acellular scaffold was studied. Generally, the cell seeding onto the culture dish is achieved by a static incubation of the cell suspension in about a few hours. It is not difficult to seed the cells on the inner surface of a tubular body like blood vessels by the rolling of the body at a low speed continuously or intermittently in the cell suspension. Since the heart valve has relatively complicated three dimensional cusp surfaces, it is not easy to seed the cells uniformly and completely on them by a simple roller culture. Some research groups have reported pulsatile circulatory bioreactors and/or multi-rotation axes bioreactors for cell seeding and expansion on the heart valve scaffolds (Zeltinger J, et al. 2001, Laube and Matthaus 2001, and Hildebrand DK, et al. 2004). Prof. Umezu and his group have reported a combined bioreactor with multi-axes and a circulation culture for decellularization and endothelial cell seeding sequentially (Iwasaki K. 2004). We have developed a simple double axes roller culture system using a bottle roller for the cell seeding on the valve scaffold and a circulation culture system using a blood pump and gas exchanger for their expansion. Porcine endothelial cells were isolated from the femoral artery of a future recipient by collagenase digestion. After 3 weeks in vitro expansion of the cells, the endothelial cells were suspended and seeded by the roller culture system for 4 hrs. The cells were then expanded in the circulation culture system for 5 days. The autologous endothelial cells were well seeded onto the three dimensional surfaces of the PowerGraft heart valve by the culture systems (Fig. 5).

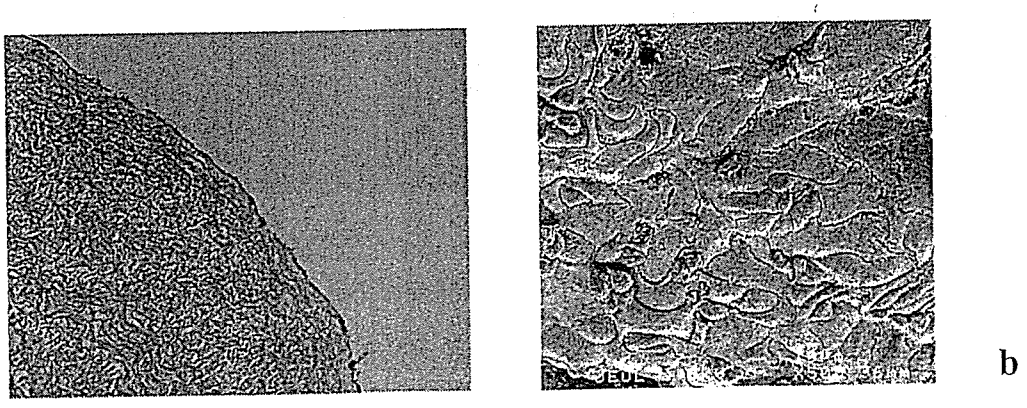

Fig. 5a b. Endothelial cells seeded on the PowerGraft heart valve by the roller and circulation culture systems. **a** H-E. **b** SEM.

Preliminary Animal study of Power Graft

Some research groups have already put their TE heart valves to clinical trials. The allogeneic transplantation study of the PowerGraft aorta and pulmonary heart valve was continued in a porcine model as a pre-clinical study. The morphological and histological changes after the implantation were evaluated in the aorta model without cell seeding, because it seemed the strength and calcification of the acellular tissue in the artery was more critical than the pulmonary tissue. The decellularized porcine aorta was implanted at the descending aorta in the Clawn miniature pig through a left thoracotomy. Surgery was carried out with single clamp technique and the animals were sacrificed at 4 weeks and 12 weeks after the implantation. The explanted grafts were examined histologically and immunohistologically. There was no dilatation and no aneurysmal change and the explanted grafts showed no macroscopic abnormality including their anastomoses. The inner surface was smooth and had no thrombus formation. Cell infiltration was identified at 4 weeks dominantly on the outer side and intimal thickening was observed at 12 weeks. The luminal surfaces of the aorta were completely covered with endothelial cells at 4 weeks after the implantation (Fig. 6). These results are very encouraging to produce durable and safe TE heart valves.

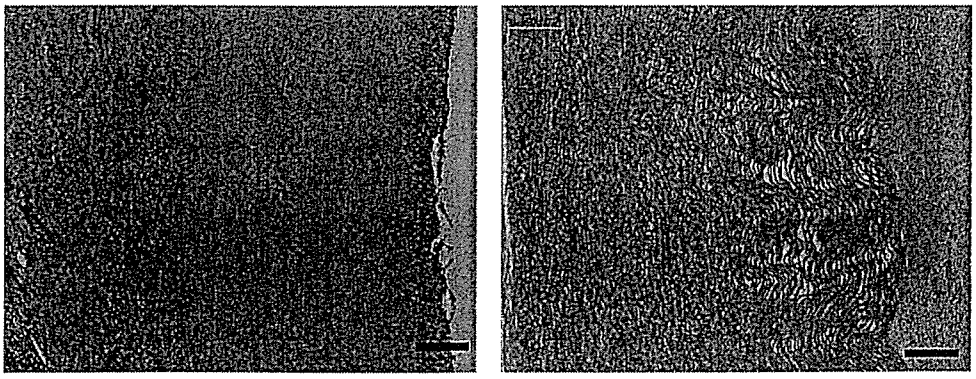

Fig. 6a. b. Explanted aortas decellularized by PowerGraft technology without cell seeding after the allogeneic transplantation in the miniature pig model. **a** 4 weeks and **b** 12 weeks after the transplantation. Left side is the outer side of the implanted graft. Scale bars are 200 μm.

Conclusion

There have been a lot of medical devices developed that still require innovation in many areas and are unable to give growth activity to the current artificial devices. In the heart valves, limitations on homograft valve availability require the need for a better clinical option for the patient and surgeon especially with respect to the pediatric patients because of the limited outcomes of current artificial heart valves. We are developing custom-made tissue transplantation in which patient's autologous cells are seeded on and in the appropriate scaffold for defective tissues of heart valves, blood vessels, pericardium, trachea, esophagus, and dura mater. Our novel decellularization method of PowerGraft was developed to produce a safe bioscaffold by ultrahigh pressure treatment of the CIP and washing under microwave irradiation. Porcine cells and PERV were removed completely from the animal tissues in a short period by the CIP of 980 MPa without changing the biomechanical properties. These findings suggest the tissues treated with CIP can be used as a safe bioscaffold, even if based on xenogenic tissues that have risks of unknown animal related diseases. We are currently studying autologous cell seeding on and in the scaffold prepared by PowerGraft technology and their applications in animal experiments. These acellular tissues are going to be put into clinical study in the near future. The TE heart valves might be substituted for the current artificial heart valves in the future.

Acknowledgement

This study was supported by the Research Grants from Ministry of Health, Labour and Welfare and Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Bader A, Schilling T, Teebken OE, Brandes G, Herden T, Steinhoff G, Haverich A (1998) Tissue engineering of heart valves--human endothelial cell seeding

of detergent acellularized porcine valves. Eur J Cardiothorac Surg 14(3):279-84

Booth C, Korossis SA, Wilcox HE, Watterson KG, Kearney JN, Fisher J, Ingham E (2002) Tissue engineering of cardiac valve prostheses I: development and histological characterization of an acellular porcine scaffold. Heart Valve Dis 11(4):457-62

Dohmen PM, Lembcke A, Hotz H, Kivelitz D, Konertz WF (2002) Ross operation with a tissue-engineered heart valve. Ann Thorac Surg 74(5):1438-42

Elkins RC, Goldstein S, Hewitt CW, Walsh SP, Dawson PE, Ollerenshaw JD, Black KS, Clarke DR, O'brien MF (2001) Recellularization of heart valve grafts by a process of adaptive remodeling. Semin Thorac Cardiovasc Surg 13(4 Suppl 1):87-92

Hayashi R (2002) High pressure in bioscience and biotechnology: pure science encompassed in pursuit of value. Biochim Biophys Acta 1595(1-2):397-9

Hildebrand DK, Wu ZJ, Mayer JE Jr, Sacks MS (2004) Design and hydrodynamic evaluation of a novel pulsatile bioreactor for biologically active heart valves. Ann Biomed Eng 32(8):1039-49

Laube HR, Matthaus M (2001) A new semi-automatic endothelial cell seeding technique for biological prosthetic heart valves. Int J Artif Organs 24(4):243-6

Magre S, Takeuchi Y, Bartosch B (2003) Xenotransplantation and pig endogenous retroviruses. Rev Med Virol 13(5):311-29

Schenke-Layland K, Opitz F, Gross M, Doring C, Halbhuber KJ, Schirrmeister F, Wahlers T, Stock UA (2003) Complete dynamic repopulation of decellularized heart valves by application of defined physical signals-an in vitro study. Cardiovasc Res 60(3):497-509

Simon P, Kasimir MT, Seebacher G, Weigel G, Ullrich R, Salzer-Muhar U, Rieder E, Wolner E (2003) Early failure of the tissue engineered porcine heart valve SYNERGRAFT™ in pediatric patients. Euro J Cardiothorac Surg 23:1002-6

Teebken OE, Puschmann C, Aper T, Haverich A, Mertsching H (2003) Tissue-engineered bioprosthetic venous valve: a long-term study in sheep. Eur J Vasc Endovasc Surg 25(4):305-12

Zeltinger J, Landeen LK, Alexander HG, Kidd ID, Sibanda B (2001) Development and characterization of tissue-engineered aortic valves. Tissue Eng 7(1):9-22

第3章　生体適合性評価法

岸田晶夫*

1　はじめに

医用材料の基本条件[1~3]は，最小限の生体機能代行性と生体安全性（非毒性），および生体適合性の3つである。前2者は不可欠条件であり，今日のほとんどすべての材料はこれらの最低レベルを満足している。一方，生体適合性は必ずしも必須ではないが，優れた特性を持っている材料でも，生体適合性が不十分なために医療に応用できない場合もあると言われ，これまでに多くの研究が行われてきた。しかしながら，今日では医用材料の生体適合性についての議論は拡散している感がある。これは開発される医療材料が，従来の汎用的なものからより合目的なものに特化されているためである。このような現状をふまえて，生体適合性についての考え方から新しい評価法について紹介する。

2　生体－材料間の反応と生体適合性

医療用材料（バイオマテリアル）は，損傷あるいは失われた組織の機能を代行・改善するために，生体内に設置されたり，チューブと連結されて生体外で器官の機能代行をしたりする。バイオマテリアルを用いるためには，それが生体内であれ生体外であれ，最初に生体組織を傷つけなければならない。生体組織が傷つけられる，または破壊されると，周辺の細胞は創傷治癒反応を開始する。創傷への急性反応は炎症反応である。白血球は創傷部位において，侵襲と感染に対して防御反応を起こす。損傷組織によって放出される化学メディエータが白血球を集合させ，炎症反応の引き金となる。炎症のこの第1段階は急性炎症と呼ばれる。この段階にかかわる白血球の大部分は好中球である。次の第2段階は慢性炎症である。単球と呼ばれる白血球は炎症部位に移動し，成熟してマクロファージとなる。治癒に不利な異物反応がなく，さらに感染が全くない場合には，炎症反応は軽微なものとなる。その後，創傷治癒が開始され肉芽組織が形成される。移植されたバイオマテリアルがマクロファージと異物巨細胞で覆われるようになるとき，一般的には肉芽組織形成から線維組織による被包化が進行し，最終的にカプセル化によって周辺組織と隔

* Akio Kishida　東京医科歯科大学　生体材料工学研究所　教授

離される。肉芽組織やカプセル化などの創傷治癒部が形成される程度は，周囲の組織と注入される材料，その表面特性，および細胞の再生能に依存する。この正常な治癒過程が進行する場合，生体内に移植されたバイオマテリアルを「生体適合性である」と呼ぶことができる。一方，材料の化学的，物理的特性あるいは移植部位での材料の動きによって慢性炎症が引き起こされる可能性もある。それらの反応は細胞の損傷をもたらすので，炎症反応のための引き金が引き続けられその結果，炎症が持続する。このように，「生体適合性」は材料と生体組織の間の反応を決定するための用語であり，かつ生体システムとバイオマテリアルの相互作用の結果であると説明される。

3 生体適合性の定義

生体適合性は定義が難しいが，通常，「材料が特定の用途について，適切な宿主反応の範囲内で効果を発揮する能力のこと」と定義されている[4]。また，他の表現では，「生体に対して全く影響を与えない（少なくとも観察している時間範囲内で）物質は生体適合性である」と表される[5]。また，我々がどのようにバイオマテリアルを使用したいかによって，バイオマテリアルの生体適合性の定義は異なる。「細胞接着性」について例をあげれば，バイオマテリアルが組織に植えつけられるのであるなら，そのバイオマテリアルに対して細胞が接着し，増殖できることが生体適合性の要件であると考える。一方，血液に接触するのであれば，血液細胞が接着しないことが生体適合性の要件である場合と，早期に内皮細胞の接着と増殖が必要である場合の相反する特性のいずれもが生体適合性の要件となる場合もある。他の見方をすると，バイオマテリアルと組織界面が使用されている期間内で安定しているとき，そのバイオマテリアルは生体適合性であると言える。界面（インターフェイス）が不安定であると，刺激，炎症，損傷，免疫源性，発熱源性，毒性，変異源性または発癌性などの反応の惹起が懸念され，そのバイオマテリアルは「生体非適合性である」と定義される。このように種々の生体適合性についての考え方があり，その評価法・試験法については，それぞれの状況に応じたものを選択する必要がある。

4 生体適合性試験法

一般に，細胞や器官などで構成される生体システムと関連して用いられるいずれの分子，材料またデバイス（機器）も，臨床応用する前に生体システムへの効果を試験しなければならない。実際に市販される（されている）バイオマテリアルに関しては，生物学的適合性の試験は高度に規制されている。しかしながら，この試験では「生体適合性」という単語は「無毒性」と同じ意

体で使用される。一般に，生体適合性試験を実行するための方法のガイドラインが，ASTM 特別部会 F04 や国際標準化機構（ISO）の第 194 技術部会（TC194）による規格の中で議論されている。ISO 10993 規格に基づいて，米国の食品医薬品局（FDA）は米国とヨーロッパでの主な試験法を統一した。また，日本の当局は医療機器の毒物学的な試験試行のための国際向けガイドラインを発行している。このドキュメントは Medical Materials の Basic Biological Tests と Devices 3 のための Guidelines として非公式の翻訳で利用可能である[6]。それは ISO 10993 と構成と内容について共通部分をもっている[7]。

試験法としては，生体外（*in vitro*）と生体内（*in vivo*）で評価する二法がある。通常は *in vitro* 試験から実施する。いくつかの試験法で，材料と細胞が直接に接触した状態で試験される。直接接触法の利点は，簡便であり，生体内移植において細胞が材料に接触している状況のための最高のモデルであるということである。他の試験法では，材料は最初に液体で抽出され，その抽出液について試験される。用いられる状況（臨床）または材料の特質によって，使用される抽出法は異なっている。一般に，2 種の溶媒（1 つは極性溶媒，もう 1 つは非極性溶媒）が使用される。これらの試験は，細胞毒性，免疫系への刺激（特にアレルギー），慢性炎症の惹起，血液と血液成分への影響，および変異源性と腫瘍形成を含む遺伝因子への効果について検討するようにデザインされている。

In vitro 試験で問題がなかった場合は，続いて *in vivo* 試験が必要である。これは動物を用いて安全性評価と機能評価を行うものである。硬組織バイオマテリアル（人工関節や人工骨）を試験するためには，試験に必要な十分量の緻密骨を得るために犬や羊が必要になる。他のバイオマテリアルに関しては，初期の通常の移植部位は皮下である。マウス，ネズミおよびモルモットを使用し，線維性カプセルの厚さ，バイオマテリアル周辺の炎症などが調査される。また，安全性評価に特化した生物学的安全性評価は，すべての新しいバイオマテリアルで必要であり，これまでに用いられているバイオマテリアルでも新しい目的で使用される場合は必要となる。

5　生体適合性試験の新局面

バイオマテリアル－生体組織間の相互作用はさまざまな疾病の状態と治療に関連している。最近，バイオマテリアル－生体組織間の相互作用は，再生医工学や生体工学と呼ばれる領域で注目されている。この領域では，バイオマテリアルは，恒久的足場材料，生体内分解性足場材料，ドラッグデリバリー用デバイス，およびバリヤー材料として利用される。生体適合性の研究の先端領域では，研究者は生体適合性の新局面に直面する。バイオマテリアルが生体内でどのように働くかを知ることができれば，生体適合性の分子論的な背景が明らかになり，これを基盤に新しい