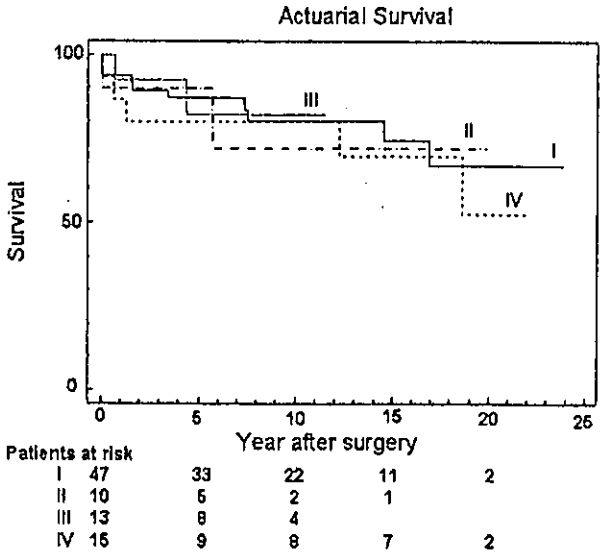

Figure 6. Actuarial survival in each group.

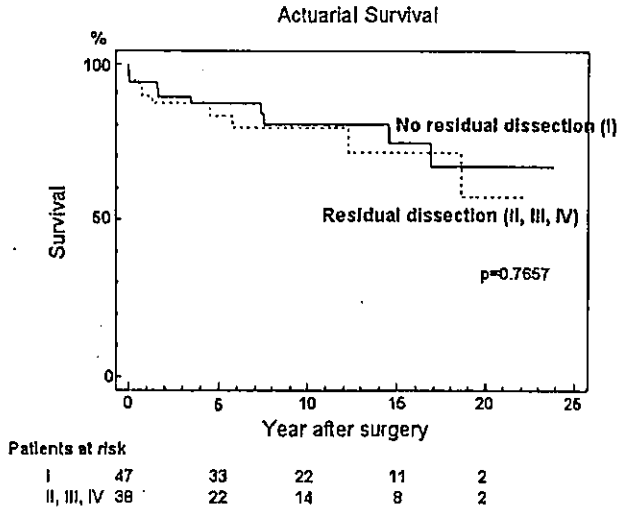

Figure 7. Actuarial survival in patients with and without residual dissection, showing no significant difference ($P = .7657$) between groups.

The introduction of aortic root replacement with a composite graft by Bentall and De Bono[11] in 1968 significantly improved the surgical results in such cases. In recent years, elective surgery has been performed with an operative risk below 5% to provide a full recovery and a normal lifestyle.[5-7,12] In our series of operations on patients with Marfan syndrome, the mortality among patients with aortic root replacement between 1990 and 2002 was 4.2% (2/48), which was almost half (8.1%) that in earlier experiences between 1977 and 1989. Moreover, the life expectancy of patients with Marfan syndrome has significantly improved (actuarial survivals were 80% at 10 years and 61% at 20 years).

However, the abnormal aortic tissue in Marfan syndrome requires multiple surgical reconstructions, and the quality of life of patients with Marfan syndrome is significantly restricted by repeated operations. Of all 85 patients in this study, 28 (32.9%) required a total number of 47 subsequent staged operations. This was more common among patients with residual aortic dissection after the initial operation. In this study, the actuarial freedom from reoperation among patients with residual dissection (group II, III, IV) was significantly lower than that among patients without residual dissection (group I). The linearized rate of reoperation was also significantly higher. Unfortunately, this trend of reoperation was immutable even in patients who received concomitant total arch replacement for associated type A dissection. Among patients with aortic dissection in the aortic arch, there was no significant difference in the actuarial freedom from reoperation ($P = .177$) and the linearized rate of reoperation ($P = .900$) between patients with concomitant total arch replacement (group III) and patients with aortic root replacement alone (group IV). Indeed, more than 60% of patients with Marfan syndrome require a reoperation within 10 years, irrespective of whether the total aortic arch is replaced or not. These results showed that concomitant total arch replacement was therapeutic but not curative, because multiple reentries in the fragile dissected septum in Marfan syndrome disturbed the thrombosed closure of the residual false channel.[13-15] On the basis of these results, controversy still continues regarding whether concomitant total arch replacement is necessary for a dissecting aortic arch.[16,17] Concerning the second-stage operation, however, it is generally accepted that replacement of the descending thoracic aorta through left thoracotomy is preferable to total arch replacement through median resternotomy. Additionally, regarding the staged operation for entire aorta replacement, concomitant total arch replacement is more advantageous than aortic root replacement alone. Among our patients, 4 patients in group III had a complete aortic reconstruction with a total of 12 operations, whereas 3 patients in group IV had complete aortic reconstruction with a total of 12 operations (Figure 2). We therefore recommend total arch replacement for type A dissection involving the aortic arch simultaneously with aortic root replacement for annuloaortic ectasia as long as the patient's condition permits this.

Another point highlighted by this study is the late fate of the residual intact arch after aortic root replacement with abnormal tissue, that is, the potential risk of dissection or rupture in the future. This is a key to answering the primary question of whether the intact aortic arch should be replaced prophylactically and aggressively at the time of aortic root replacement for annuloaortic ectasia.

In general, the cause of aortic dissection is the process of injury and repair of the aortic media by the turbulence of blood flow.[18] The initial intimal tear of a dissection is most frequently situated within the first few centimeters of the ascending aorta.[19] In Marfan syndrome, aortic dissection sometimes occurs in a normal-sized ascending aorta, although the aortic root and sinus of Valsalva are enlarged.[8] On the basis of our results, we propose the possible mechanism of a tear in ascending aorta among patients with Marfan syndrome as follows. Turbulence in the ascending aorta is exacerbated by both the velocity of ejected blood and the interface between the ejected blood and the relatively stagnant blood. However, in a normal aortic root, the position of the leaflets helps to reduce turbulence by masking the dilated sinuses and producing a uniform diameter above the ventriculoarterial junction when blood is ejected through the valve orifice.[20-24] In contrast, in a gourdlike aortic root, which is typical of annuloaortic ectasia in Marfan syndrome, this mechanism is not effective. The high-velocity flow of ejected blood reaches the ascending aortic wall with medial degeneration, and turbulence occurs at the junction between the ascending aorta and the dilated sinus of Valsalva because the leaflets cannot mask the dilated sinus of Valsalva (Figure 8).[25] Another factor in dissection is the different tensile strength of the aortic wall itself, which depends on the content of elastic fibers and collagen. The contents of elastic fibers and collagen differ between the ascending aorta and the sinus of Valsalva[26-30] and also between the inner and outer layers of the aortic wall itself. This leads to the "breaking point" of the internal layer in the ascending aorta during aortic dilatation.[21] Therefore these two major factors will be eliminated by composite graft replacement of the dilated sinus of Valsalva and the proximal ascending aorta. This hypothesis is supported by the long-term results of patients with an intact arch in our study. The incidence of new dissection in the residual intact arch after aortic root replacement was extremely low; only 2 of the 53 patients who survived initial hospitalization with an intact arch underwent subsequent total arch replacement for the onset of arch dissection, whereas 4 of the 14 patients who survived initial hospitalization with dissection in the aortic arch underwent subsequent total arch replacement. These results show that prophylactic replacement of the intact aortic arch is not necessary at the time of aortic root replacement for annuloaortic ectasia, because aortic root replacement itself plays a prophylactic role in aortic dissection.

The major limitation of our study was that the time scale of this study ranged through 22 years. During this period mortality and morbidity in aortic surgery were clearly improved by refinements in surgical technique and perioperative management. Additionally, a large proportion of the patients required ongoing treatment and follow-up. Another limitation of this study was that the numbers of patients in each group were too small to ascertain any statistically significant difference between groups for operative mortality or the necessity for reintervention.

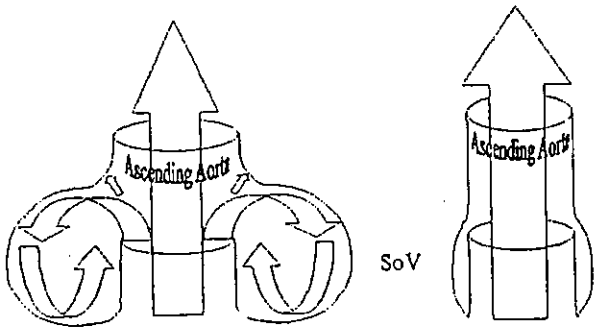

Figure 8. ***Right,*** **Normal aortic root. Position of leaflets helps to reduce turbulence by masking dilated sinus to produce uniform diameter above ventriculoarterial junction when blood is ejected through valve orifice.** ***Left,*** **Typical annuloaortic ectasia in Marfan syndrome. High-velocity flow of ejected blood reaches ascending aortic wall with medial degeneration, and turbulence occurs at junction between ascending aorta and dilated sinus of Valsalva** ***(SoV)*** **because leaflets cannot mask dilated sinus.** ***Arrows*** **indicate blood flow. Sinus of Valsalva has less elastic fiber than collagen; ascending aorta has more elastic fiber than collagen.**

In conclusion, the incidence of new dissection in the residual intact arch after aortic root replacement is extremely low, because aortic root replacement may remove the factors provoking dissection. According to our results, prophylactic replacement of the intact arch in Marfan syndrome is not necessary during aortic root replacement for annuloaortic ectasia.

ACD

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Discussion

Dr Nicholas Kouchoukos *(St Louis, Mo).* In their study, Tagusari and colleagues have focused on the question of whether the aortic arch should be replaced at the time of replacement of the dilated aortic root in patients with Marfan syndrome. Among the 57 patients with an intact arch at the time of aortic root replacement, only 2 required subsequent arch replacement for new-onset arch dissection. The actuarial freedom of reoperation in this group was 90% at 10 years. These observations are in agreement with the results of other large series of patients with Marfan syndrome and an intact arch who have undergone only aortic root replacement. The aggregate experience strongly suggests that replacement of the arch is not indicated in this setting.

What is less clear is whether the aortic arch should be replaced at the time of aortic root replacement in patients with acute or chronic type A dissection. Albeit the number of patients with dissection in this series was small, 29 patients, no difference in survival or in freedom from any operation was observed at 10 years between the 15 patients undergoing only aortic root replacement and the 13 patients who had aortic root and simultaneous arch replacement. However, the rate of reoperation on the aortic arch was significantly higher among the patients with dissected arch who underwent only aortic root replacement than among the patients with an intact arch. This suggests that replacement of the dissected arch may be advantageous.

Only 10 of the 46 patients with dissection in this series had acute dissection. Tagusari and colleagues did not examine outcomes in this subgroup, presumably because of small numbers. However, the decision to replace the aortic arch in the presence of acute dissection may be associated with higher risk than if the dissection is chronic.

I have several questions for Dr Tagusari. First, on the basis of your findings, what is your current strategy for management of patients undergoing aortic root replacement who have a type A dissection?

Dr Tagusari. We perform aortic root replacement with composite graft and total arch replacement.

Dr Kouchoukos. Do you manage the patients with acute dissection in this setting any differently than you manage the patients with chronic dissection?

Dr Tagusari. In general, the patient with Marfan syndrome who has an aortic dissection is young. Accordingly, we should perform total arch replacement simultaneously to save further operation.

Dr Kouchoukos. Do you recommend complete aortic arch replacement in the setting of acute dissection?

Dr Tagusari. Yes.

Dr Kouchoukos. A valve-sparing procedure was performed in 17 of the 86 patients in the series. How much of the ascending aorta was replaced in these patients? Do you believe, from your experience to date, that this is a durable procedure in patients with Marfan syndrome?

Dr Tagusari. For the patient with Marfan syndrome, I prefer a composite graft replacement to valve-sparing operation, especially a remodeling procedure, because in our histologic findings the aortic valve showed concentric layering of collagen fibers mixed with glycosaminoglycans. This means severe degeneration of the leaflet itself. Actually, 4 of the 17 patients who underwent valve-sparing operations (2 of 13 reimplantations and 2 of 4 remodelings) needed aortic valve replacement.

Total Arterial Off-Pump Coronary Artery Bypass Grafting for Revascularization of the Total Coronary System: Clinical Outcome and Angiographic Evaluation

Osamu Tagusari, MD, Junjiro Kobayashi, MD, Ko Bando, MD, Kazuo Niwaya, MD, Hiroyuki Nakajima, MD, Takeshi Nakatani, MD, Toshikatsu Yagihara, MD, and Soichiro Kitamura, MD

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Background. We assessed the clinical outcome and conducted an angiographic study of total arterial off-pump coronary artery bypass grafting for revascularization of the total coronary system.

Methods. Of 382 consecutive patients who underwent off-pump coronary artery bypass between April 2000 and December 2002, 235 patients (193 men and 42 women, mean age 66 ± 9 years) with three-vessel disease underwent off-pump coronary artery bypass with all arterial grafts. A total of 872 vessels were bypassed (average number of grafts 3.7 ± 0.8). The internal thoracic arteries, radial arteries, and gastroepiploic arteries were used for revascularization of 306, 542, and 24 coronary arteries, respectively. Two hundred twenty-five patients underwent revascularization with composite grafts that were connected to the in situ internal thoracic artery (Y configuration 181, I configuration 55, K configuration 27, X configuration 3, T configuration 1); 10 patients underwent revascularization with all in situ grafts.

Results. Three (1.3%) hospital deaths and 1 late death occurred. There were no occurrences of clinical underperfusion syndrome or new intraaortic balloon pump insertion. Cerebral infarction occurred in 2 patients (0.8%). Early postoperative angiography was performed on 833 grafts in 223 patients (95%); the overall patency rate was 98%. Stratified by coronary distribution, the patency rate was 99% (218/221) in the left anterior descending artery, 97% (84/87) in the diagonal artery, 99% (70/71) in the obtuse marginal artery, 98% (262/268) in the posterolateral artery, 98% (167/170) in the posterior descending artery, and 100% (16/16) in the right coronary artery.

Conclusions. Total arterial off-pump coronary artery bypass yielded good clinical results and an excellent patency rate of revascularization for the total coronary system.

(Ann Thorac Surg 2004;78:1304–11)

Recently, off-pump coronary artery bypass (OPCAB) operation has been widely used and adopted by many surgical groups due to the accumulation of experience and improvement of surgical techniques and stabilization devices [1–4]. However, a major concern still exists in the accuracy of the coronary anastomosis performed with arterial grafts on a beating heart. Another concern is the ability to perform a complete revascularization in patients with multiple-vessel disease using this technique. The aim of this study was to demonstrate the feasibility of performing total arterial revascularization for the total coronary system with this approach.

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Material and Methods

Patient Selection

Between April 2000 and December 2002, OPCAB was performed in 451 patients, except for 2 patients who were converted from off-pump to on-pump coronary artery bypass grafting (CABG) because of hemodynamic instability during anastomosis of the left anterior descending artery (LAD). Three hundred thirty-two patients had three-vessel disease. Of these patients, 235 patients underwent revascularization with all arterial grafts. The preoperative characteristics of the patients are summarized in Table 1.

Conduit Selection

Basically, we used the radial artery (RA) composite graft in combination with one or both internal thoracic arteries (ITA) for revascularization of the total coronary system. In particular, single ITA and the RA as a composite graft were used for elder patients or patients with poor risks. The gastroepiploic artery (GEA) was harvested in pa-

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Abbreviations and Acronyms

CABG = coronary artery bypass grafting
CK-MB = creatine kinase-MB
DG = diagonal branch
ECG = electrocardiogram
GEA = gastroepiploic artery
ITA = internal thoracic artery
LAD = left anterior descending artery
LITA = left internal thoracic artery
OM = obtuse marginal
OPCAB = off-pump coronary artery bypass
PD = posterior descending
PL = posterolateral
RA = radial artery
RITA = right internal thoracic artery

tients whose RA was not available because of a positive Allen's test in the bilateral forearms or chronic renal failure (serum creatinine > 1.5 mg/dL).

Because possible stenosis of the subclavian artery and celiac artery may be a cause of concern, we routinely evaluate by preoperative imaging such as computed tomography, magnetic resonance angiography, or angiography.

Preparation of the Conduits

A conventional, semiskeletonized method was used for the dissection of the ITAs. The RA was harvested from the nondominant arm using an ultrasonic dissection technique [5]. All arterial conduits were wrapped in a sponge soaked with a solution of papaverine hydrochloride. After administration of heparin (1.5 mg/kg), the arterial grafts were divided. A mixture of blood and a solution of papaverine hydrochloride was gently injected into the lumen of the RA to prevent spasm. The left internal thoracic artery (LITA) was generally used for revascularization of the LAD, or blood source of the RA as various configurations of the composite graft. When the GEA was harvested, it was used as an in situ graft to the posterior descending (PD) or/and posterolateral (PL) artery. The right internal thoracic artery (RITA) was anastomosed to the LAD as an in situ graft or connected to the RA for extension as an I or Y graft. Y configuration was the most common composite graft for multivessel revascularization. A K configuration was used when the diagonal branch (DG) ran parallel to the LAD. The advantage of the K graft is that it spares the length of RA, because a long RA segment is required to avoid kinking when the RA is used as a Y composite graft sewn on the DG that runs parallel to the LAD in a side-to-side fashion (Fig 1). With this K graft, the other end of the RA extends to the PD with a few side-to-side anastomoses (Fig 2). Sequential bypass of the LITA to DG and the LAD was also performed in our series. However, side-to-side anastomosis between the LITA and the DG is technically demanding on the beating heart when the LITA is small. As for the X graft, the RA can extend fully around the heart from the DG to the right coronary artery (Fig 3). All composite grafts except the I configuration were arranged before the distal anastomoses. In the I configuration, the RA was connected to the RITA after the distal anastomoses to adjust the length of the RITA.

Table 1. Preoperative Characteristics (n = 235)

	Number	Percent
Age (y)		
Mean	66.2 ± 8.7 (range 42–84)	
≥65 y and ≤74 y	99	42
≥75 y	45	19
Male/Female	193/42	
LVEF < 0.35	40	17
Preoperative IABP	7	3
Acute MI	4	2
Emergency case	7	3
Reoperation	7	3
History of PCI	46	20
Diabetes mellitus	83	35
Cerebrovascular disease	51	22
Chronic renal failure	8	3
Hemodialysis	6	3
COPD	7	3
Higgins Score		
Mean	3.3 ± 2.8	
≥5	61	26

COPD = chronic obstructive pulmonary disease; IABP = intra-aortic balloon pumping; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PCI = percutaneous coronary intervention.

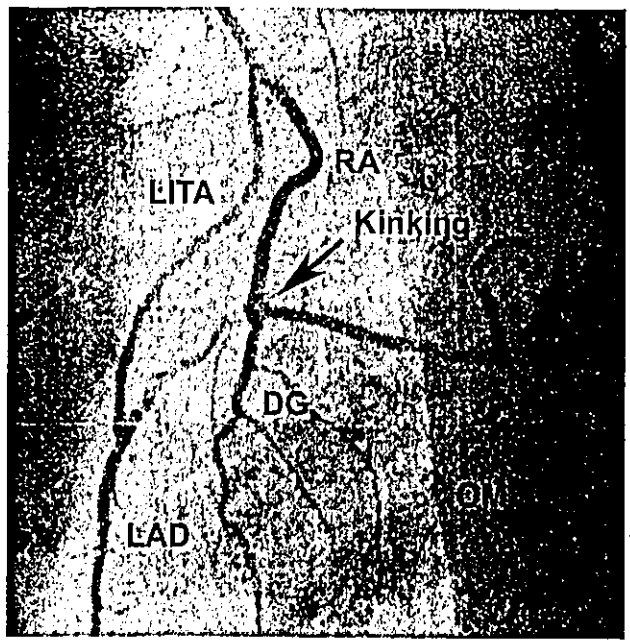

Fig 1. Y composite graft. The radial artery (RA) shows kinking when the RA as a Y composite graft is sewn on the diagonal (DG) that runs parallel to the left anterior descending artery (LAD) in a side-to-side fashion. (LITA = left internal thoracic artery; OM = obtuse marginal.)

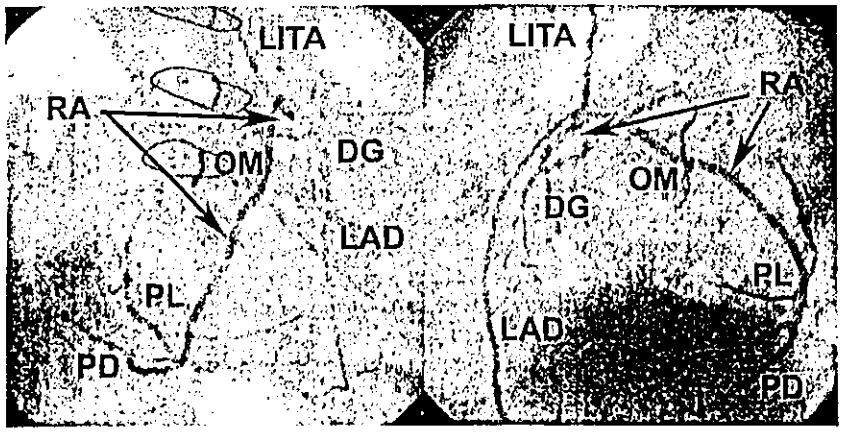

Fig 2. K composite graft. The advantage of the K graft is sparing the length of the radial artery (RA), when bypass is necessary for the diagonal (DG) that runs parallel to the left anterior descending artery (LAD). (LITA = left internal thoracic artery; OM = obtuse marginal; PD = posterior descending; PL = posterolateral.)

Surgical Procedure

A standard median sternotomy was used in all patients. The pericardium was opened and deep pericardial retraction sutures were made. Proper positioning and stabilization of the heart were achieved by pericardial sutures and suction stabilizers (Medtronic Octopus, Starfish, Medtronic, Inc, Minneapolis, MN) with rotation of the operative table. Transesophageal echocardiography and pulmonary artery pressure monitoring were performed to check mitral regurgitation due to extensive left ventricular geometric change and right ventricular outflow obstruction due to the right ventricular geometric change. Only transient proximal compression of the target vessel to control the coronary blood flow was accomplished with a silicone rubber loop. The heart rate and blood pressure control were obtained with diltiazem, landiolol hydrochloride, and norepinephrine. After coronary arteriotomy, an intraluminal shunt was inserted to maintain the coronary blood flow and bloodless operative field. A carbon dioxide saline-blower was also used to eliminate excessive blood from opened coronary vessel. The coronary artery was anastomosed in the order of LAD, DG, obtuse marginal (OM), and PL to the PD. The sequential anastomosis technique is a diamond-shaped (90° crossing) method with the RA, and usually a parallel method with in situ arterial grafts. To prevent arterial spasm, diltiazem (0.5 to 1.0 μg/kg) or nicardipine (0.1 to 0.2 μg/kg) was infused intraoperatively and during the first 16 hours after the operation. Diltiazem (100 to 200 mg/d) or amlodipine (2.5 to 5.0 mg/d) was then prescribed orally in conjunction with aspirin (162 mg/d) from the next morning.

Angiographic Study

In 223 (94.9%) patients, informed consent was obtained and follow-up angiography was performed before hospital discharge by cardiologists. By means of the Cardiovascular Measurement System (QCA-CMS, version 4.1; Medical Imaging System, Leiden, The Netherlands), the stenotic percentage of anastomosis was calculated by comparing the diameter of anastomosis with that of the proximal portion of the graft at the view of the minimum lumen. A percentage stenosis of less than 50% was assessed to be patent [6, 7].

Data Collection and Follow-Up

We retrospectively reviewed the data from the operation notes, anesthesia records, clinical histories, laboratory investigations, and cardiac catheterization. This retrospective study was approved by the Internal Review Board of the National Cardiovascular Center. Follow-up data were collected from the medical records of outpatient visits and correspondence with referring physicians.

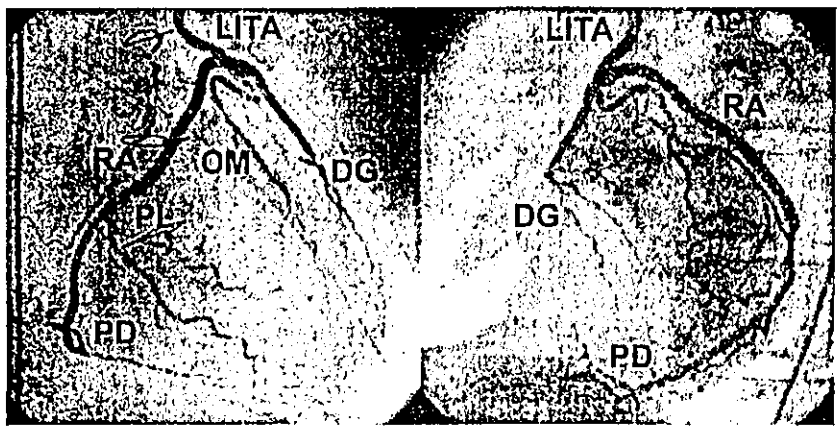

Fig 3. X composite graft. In an X graft, the radial artery (RA) can extend fully around the heart from the diagonal (DG) to the posterior descending (PD) artery. (LITA = left internal thoracic artery; OM = obtuse marginal; PL = posterolateral.)

All clinical characteristics were accumulated as a computerized database and analyzed.

Statistical Methods

All values are expressed as the mean ± standard deviation. The discrete variables were analyzed with the Fisher's exact test between two groups and Kruskal-Wallis rank test for more than three groups. Scheffé's test was performed when a significant difference was recognized in the results of the Kruskal-Wallis test. All statistical analyses were performed using the software package SPSS 10.0 for Windows (SPSS Inc, Chicago, IL). The differences were considered statistically significant when the p value was less than 0.05.

Results

Eight hundred seventy-two distal anastomoses were performed on 235 patients. The mean number of distal anastomoses was 3.71 ± 0.82 (range 3 to 7). Distal anastomosed sites were 233 LADs, 88 DGs, 73 OMs, 282 PLs, 180 PDs, and 17 right coronary arteries.

Unilateral ITA was used in 127 patients (54.0%) and bilateral ITA in 108 patients (46.0%). The RA was used as a composite graft in 232 patients (98.7%) and the GEA was used as an in situ graft in 16 patients (6.8%). Y, I, K, X, or T configuration was used for the RA composite graft in 181, 55, 27, 3, and 1 patients, respectively. Revascularization with all in situ grafts was performed in 10 patients for 40 distal anastomoses.

Angiographic Study

The patency rates according to coronary distribution, graft materials, and anastomotic fashions are shown in Table 2. The patency rates according to the configurations of the composite graft are also shown in Table 3. The overall patency rate was 98.1% (817/833). Stratified by coronary distribution, the patency rate was 98.6% in LAD, 96.6% in DG, 98.6% in OM, 97.8% in PL, 98.2% in PD, and 100% in the right coronary artery. The patency rate was 98.7% with the LITA, 95.9% with the RITA, 98.5% with the RA, and 88.0% with the GEA. The patency rate was 97.6% with the end-to-side fashion and 98.9% with the side-to-side fashion. The patency rate with GEA was significantly lower than that of the other graft materials (LITA versus GEA, $p = 0.001$; RA versus GEA, $p < 0.001$). However, no significant changes were noted in the patency rates between coronary distribution ($p = 0.846$), anastomotic fashion ($p = 0.197$), and the configuration of the composite graft ($p = 0.779$).

Five of 254 composite grafts (2.0%) showed kinking or stenosis of the LITA just proximal to the composite graft anastomosis site with the RA. Four of 303 ITAs (1.3%) showed stenosis in the middle of the vessel probably due to intraoperative injury.

Evident flow competition was observed in 36 patients. Of those, 24 RA composite grafts and 12 of the LITA distal from the anastomotic site of the composite graft to the target coronary artery were not opacified in angiography of the in situ graft, although the target coronary artery

Table 2. Patency for Bypass Distribution, Graft Material and Anastomotic Fashion

	Graft Material									
	LITA		RITA		RA		GEA		Total	
	E-S	S-S	E-S	S-S	E-S	S-S	E-S	S-S	E-S	S-S
LAD	199/201 (99.0%)	1[a]	16/17 (94.1%)	1[a]	1[a]	0	0	0	216/219 (98.6%)	2[a]
DG	7[a]	12[a]	6/7 (85.7%)	1[a]	35/37 (94.6%)	23[a]	0	0	48/51 (94.1%)	36[a]
OM	7/8 (87.5%)	0	2[a]	0	22[a]	38[a]	1[a]	0	32/33 (97.0%)	38[a]
PL	6[a]	2[a]	3[a]	0	86/88 (97.7%)	158/160 (98.8%)	6/7 (85.7%)	1/2 (50.0%)	101/104 (97.1%)	161/164 (98.2%)
PD	0	0	4[a]	0	114/116 (98.3%)	35[a]	8/9 (88.9%)	6[a]	126/129 (97.7%)	41[a]
RCA	0	0	13[a]	1[a]	2[a]	0	0	0	15[a]	1[a]
Total	219/222 (98.6%)	15[a]	44/46 (95.7%)	3[a]	260/266 (97.7%)	254/256 (99.2%)	15/17 (88.2%)	7/8 (87.5%)	538/551 (97.6%)	279/282 (98.9%)

[a] 100% of patency rate.

DG = diagonal; E-S = end-to-side anastomosis; GEA = gastroepiploic artery; LAD = left anterior descending artery; LITA = left internal thoracic artery; OM = obtuse marginal; PL = posterolateral; PD = posterior descending; RCA = right coronary artery; RITA = right internal thoracic artery; S-S = side-to-side anastomosis.

Table 3. Patency for Configurations of Composite Graft

	Configurations of Composite Graft					
	Y	I	K	X	T	Total
	171	53	26	3	1	254
DG	39/42 (92.9%)	0	28[a]	3[a]	0	70/73 (95.9%)
OM	54[a]	0	10[a]	0	0	64/64
PL	168/169 (99.4%)	45/47 (95.7%)	34[a]	3[a]	3[a]	253/256 (98.8%)
PD	100/102 (98.0%)	46[a]	12[a]	2[a]	1[a]	161/163 (98.8%)
RCA	3[a]	6[a]	0	1[a]	0	10[a]
Total	364/370 (98.4%)	97/99 (98.0%)	84[a]	9[a]	4[a]	558/566 (98.6%)

[a] 100% of patency rate

DG = diagonal; OM = obtuse marginal; PD = posterior descending; PL = posterolateral; RCA = right coronary artery.

and its anastomotic site were clearly opacified in the native coronary angiography.

Early and Late Mortalities and Morbidities

Table 4 lists the early and late complications. Early death occurred in 3 patients (1.3%) due to intracranial bleeding, aspiration pneumonia, and intestinal hemorrhage. Perioperative myocardial infarction (new Q waves in electrocardiogram [ECG], creatine kinase-MB [CK-MB] more than 50 with ECG change or CK-MB more than 70 without ECG change: normal value less than 11 IU/L in our institute) occurred in 6 patients (2.6%). Two patients had a stroke during rehabilitation, one of which occurred during the postoperative angiographic study. No clinical underperfusion syndrome was noted and new intraaortic balloon pump insertion was not necessary.

Eleven patients underwent successful percutaneous catheter intervention (balloon angioplasty or coronary stenting), although they were asymptomatic. Balloon angioplasties were performed in the stenosis of the LITA just proximal to the connection of the Y composite graft (Fig 4) in 3 patients, in the stenosis of the middle of the LITA in 4 patients, and in the stenosis of the native coronary artery in 2 patients. Coronary stenting was performed in the stenosis of the RITA just proximal to the connection of the I composite graft (Fig 5) in 1 patient and the stenosis of the middle of the LITA in 1 patient. One patient underwent redo OPCAB before discharge due to occlusion of the LITA to the LAD.

One patient died suddenly 6 months after the operation because of unknown causes. Two patients whose predischarge angiography showed anastomotic stenosis

Table 4. Early and Late Results

Results	Number
Early Results	
Hospital mortality	3 (1.3%)
Morbidity	
Perioperative MI	6 (2.6%)
Postoperative IABP	0 (0%)
Early coronary intervention	12 (5.1%)
Reexploration for bleeding	2 (0.8%)
Sternal	
Dehiscence	6 (2.6%)
Infection	0 (0%)
Cerebral infarction	2 (0.8%)[a]
Renal failure with dialysis	2 (0.3%)
Forearm	
Circulatory injury	0 (0%)
Infection	1 (0.4%)
Paresthesia	0 (0%)
Late Results	
Late death	1 (0.4%)
Cardiovascular event	
Admission for angina or CHF	3 (1.3%)
Coronary intervention	2 (0.8%)
Cerebral infarction	4 (1.7%)

[a] One occurred during postoperative angiographic study

CHF = congestive heart failure; MI = myocardial infarction.

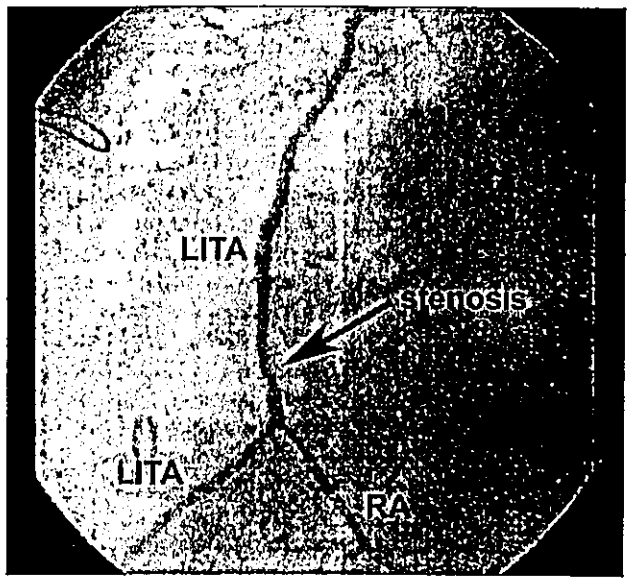

Fig 4. Stenosis of a Y composite graft. Three patients underwent balloon angioplasty, which was performed successfully in the stenosis of the left internal thoracic artery (LITA) just proximal to the connection of the Y composite graft. (RA = radial artery.)

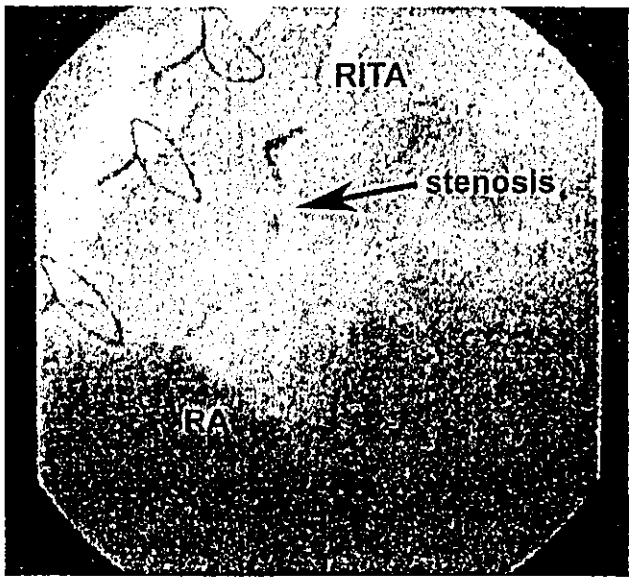

Fig 5. Stenosis of an I composite graft. Coronary stenting was performed in the stenosis of the right internal thoracic artery (RITA) just proximal to the connection of an I composite graft in 1 patient. (RA = radial artery.)

of the LAD returned to the hospital for recurrent angina and underwent successful percutaneous coronary intervention 5 months after the operation. One patient with poor left ventricular function was readmitted for congestive heart failure.

Comment

The OPCAB operation is currently considered to be a safe alternative to myocardial revascularization with cardiopulmonary bypass [1–4]. However, the ability of total arterial OPCAB for multivessel disease remains controversial. In our series, the total arterial OPCAB for the total coronary system achieved excellent patency in the early postoperative term and showed good clinical results. No significant changes were noted in the patency rates between coronary distributions ($p = 0.846$), anastomotic fashions ($p = 0.197$), and the configurations of the composite graft ($p = 0.779$), except the graft materials. The patency rate with GEA was significantly lower than that of the other graft materials (LITA versus GEA, $p = 0.001$; RA versus GEA, $p < 0.001$). The size of the GEA was variable and sometimes too small for multivessel revascularization. This finding may be the result of our performing the sequential bypass graft using small GEA. Recently, when the GEA was essential for revascularization, we have used skeletonized GEA using an ultrasonic scalpel to facilitate visual inspection [8].

Our standard technique to achieve total arterial revascularization for total coronary system is the RA composite graft in combination with one or both ITAs. The RA is used as a material for the free arterial graft because of its easier harvesting and handling. The distal sequential bypass to the coronary arteries, a thicker wall, and wider lumen compared with ITA and GEA allow meticulous anastomosis on the beating heart.

The composite graft technique appears to have advantages over the free graft that is anastomosed to the ascending aorta. First, aorta no-touch OPCAB using a composite graft reduces the incidence of neurologic complications [9, 10]. Second, a composite graft makes more efficient use of the conduit by placing the inflow close to the target coronary arteries [10–12]. Finally, the inflow of the ITA does not expose the free arterial graft to high wall stress, which may cause the early development of intimal hyperplasia [13, 14].

The composite graft technique has the pitfall of total dependence of the coronary bypass flow on the flow of the proximal ITA. We routinely evaluate the subclavian artery and the ITA by preoperative angiography, magnetic resonance angiography, or three-dimensional computed tomographic angiography, because stenosis of the proximal ITA or subclavian artery may be a cause of global ischemia. However, the adaptability of the ITA as a blood source of the arterial composite graft is still a potential risk in this technique. Multiple clinical and experimental studies have examined the suitability of the ITA as a blood source of the arterial composite graft [15–21]. From the results of positron emission tomography, Sakaguchi and colleagues [18] documented that the composite Y graft was not as effective as independent grafts for improving the coronary flow reserve soon after bypass grafting. However, most investigations have reported that the flow reserve of the proximal ITA is sufficient for a blood source of composite graft for multiple coronary revascularizations. Indeed, in the present series, there was no hypoperfusion syndrome or need for new intraaortic balloon pump insertion even in the patients whose total coronary artery system was supplied by single ITA.

In general, hypoperfusion syndrome related to the conventional CABG occurs typically 30 to 40 minutes after discontinuation of a cardiopulmonary bypass [22]. It is conceivable that the reactive hyperemia of the myocardium that presents after removal of the aortic clamp [23–25] may require greater conduit flow while the oxygen debt is repaid. This situation might produce a drastic imbalance between graft flow and myocardiac demand, resulting in the hypoperfusion syndrome [17, 26]. In our standard technique, the LAD is revascularized at first by the ITA because it is the most important coronary artery and exposure of the LAD has no major hemodynamic consequences [27]. After revascularization of the LAD, the coronary artery was anastomosed in the order of the diagonal, obtuse marginal, and posterolateral to posterior descending arterial branches with only local ischemia of the target vessel. We predicted that these techniques, which avoid intraoperative global myocardial ischemia, contributed at least partially to avoiding the hypoperfusion syndrome in our patients.

The competitive flow between the native coronary artery and bypass graft is another concern in any composite graft attached to the ITA [28]. This phenomenon was induced by graft–recipient artery mismatch. In 38

patients with composite grafts, the target coronary artery and its anastomotic site were clearly opacified in the native coronary angiography, although the bypass graft to the target coronary artery (ie, the composite graft or the distal ITA of the composite bifurcation) was not opacified in angiography of the in situ graft. Diffuse narrowing of the distal LITA, from the anastomotic site of the composite graft, was recognized in 13 patients who had competitive flow in the distal LITA to the LAD. No definite conclusion has been reached concerning the relationships between the competitive flow, diffuse narrowing, and true graft failure [29–33]. It still remains to be determined whether a particular coronary artery with a noncritical lesion should be grafted prophylactically using the arterial graft for future progression [34]. We prefer to graft to a coronary artery with moderate stenosis in a side-to-side fashion, and the termination of this conduit was to the coronary artery of severe stenosis. When the posterior descending coronary artery had only mild stenosis, we anastomosed the side of the I composite graft to that branch and the end of the I composite graft to the circumflex branches. In our study, no patient with competitive flow of bypass grafts was readmitted for angina or congestive heart failure. Although late follow-up angiography was performed in only 2 cases with competitive flow of the RA composite graft, the RA was patent at more than 1 year after the operation [10]. These results prompted us to conclude that the RA has potential as a physiologically functional arterial graft that can be recruited on demand with progression of the native coronary artery disease.

In conclusion, the total arterial OPCAB for the total coronary system achieved excellent patency in the early postoperative period and showed good clinical results. These results have prompted us to continue performing complete arterial revascularization, although long-term studies are required to provide evidence of the validity of our technique.

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DISCUSSION

DR CRAIG R. SMITH (New York, NY): One minor technical issue: you obviously carried out many side-to-side sequential anastomoses in this group. Based on your data, do you have any opinion as to whether the sequential anastomoses are better done in a diamond orientation, or in a parallel orientation, or do you do both depending on the circumstances?

DR TAGUSARI: I usually use a diamond anastomosis technique for the radial artery and a parallel fashion for the ITA and GEA.

DR HITOSHI HIROSE (Cleveland, OH): We performed a similar study for patients with three-vessel disease undergoing off-pump CABG and published the results in *Surgery* last year. Off-pump CABG provided excellent results compared with on-pump CABG and the angiographic results were competitive, as you reported today. I have several questions for the authors. First, what are the inclusion and exclusion criteria for total arterial off-pump CABG? Although the authors stated total arterial bypass is the goal for cardiac surgery, only 52% of the patients received total arterial bypass; what kind of bypass was performed in the rest of 48%? Were they received saphenous vein grafts? How do you select patients undergoing total arterial bypass? Second, only 3% of patients received a gastroepiploic artery graft. Why are the authors not using the gastroepiploic artery?

Thank you.

DR TAGUSARI: I did not include one- and two-vessel disease in this study. All the patients had three-vessel disease. The rate of complete revascularization is 100% in this series. And the next question. The size of the GEA varies; sometimes it was too small to use as a sequential bypass graft. However, recently we have used skeletonized GEA using an ultrasonic scalpel to facilitate visual inspection. We only used saphenous vein graft for the patients more than 80 years old or the patients whose radial artery was not adequate to use, such as chronic renal failure.

DR SMITH: To that question also, I think 52% was the number of patients who received OPCAB out of the total, not the percent that had total revascularization.

DR CHARLES BRIDGES (Philadelphia, PA): I have a couple of questions. One is how soon after surgery did you perform the angiogram? I know you showed one slide where it was performed two days after the operation. Another question I had was how you manage your radial artery patients after the operation, are you using nitrates, calcium blockers, and for how long are you using them? Finally, I wanted to learn a little bit more about the patient characteristics. How sick were your patients? What kind of ejection fractions did they have and how much other comorbid disease did they have? I didn't hear that or see that in your abstract, to get an idea of what kind of results we should expect. And then can you help us with the theory behind composite grafting as opposed to taking, say, a radial artery off the aorta or off of a patch of vein, et cetera, from the aorta? What is the evidence that shows us that composite grafting is theoretically superior to the more routine way, even with arterial grafts? Obviously one reason is that with a composite graft you can graft more territory than you could with just taking things off the aorta. But I was wondering if you could comment on your feeling about that.

DR TAGUSARI: We usually perform postoperative Angiography ten to fourteen days after operation. To avoid spasm of the radial artery, we use intravenous diltiazem or nicardipine until the following morning. Since then, a calcium blocker such as amlodipine has been prescribed for a long time because most of the patients have hypertension. As I have showed on the slide. In 17% of the patients, ejection fraction was less than 35% Long-term follow-up is waiting for evidence that the composite graft is superior to the coronary graft. But, when the radial artery is anastomosed directly to the aorta, it is exposed not only to high blood pressure but also to higher shear stress, which may cause the early development of the intimal hyperplasia. Additionally, we can perform aorta no-touch technique and spare the length of the radial artery and by using as a composite graft.

DR AYHAN OZDEMIR (Bursa, Turkey): The patients you had with low ventricular function, what happened after you did the coronary bypass surgery off-pump? Did their left ventricular function get better or stay the same or worse?

Thank you.

DR TAGUSARI: After the OPCAB, left ventricular function recovered significantly in most cases.

心臓病

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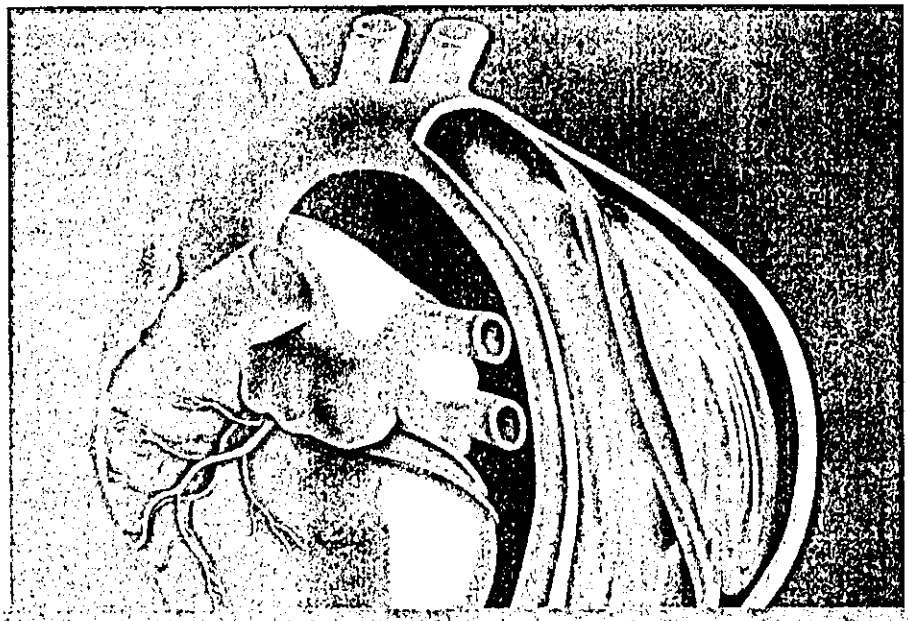

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心臓病

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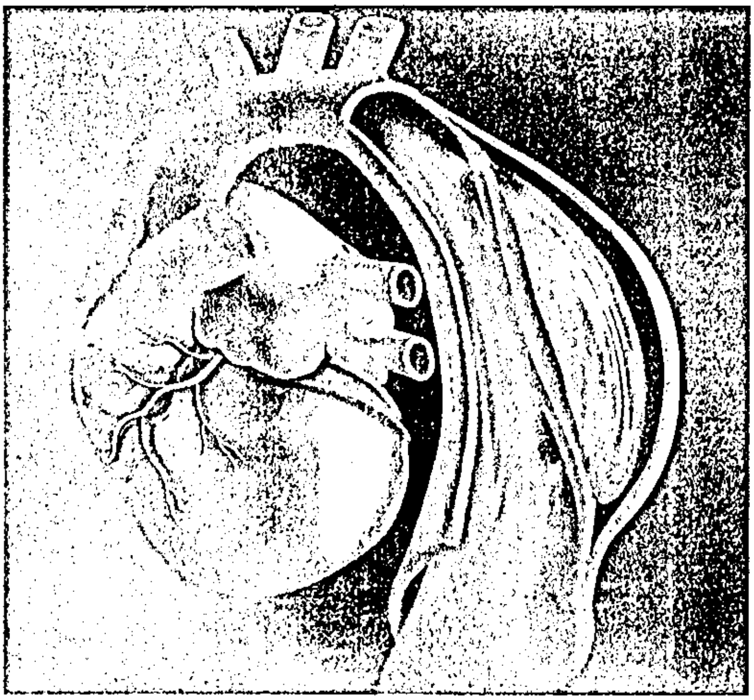

移植コーディネーター

概論

監修　日本組織移植学会

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へるす出版

巻頭言

この度，日本組織移植学会の田中秀治，篠崎尚史両理事の監修により立派な成書として『移植コーディネーター概論』が上梓された。両氏は東日本組織移植ネットワークの中心的存在であり，その設立にも深く関与されてきた専門家である。当学会の理事長としても誠に喜ばしく，かつ時期を得た重要な書であると感じている。臓器移植，組織移植，細胞移植など人からの善意の提供を受けて成り立つ，病める人への治療法には共通した基盤があり，広い領域の医療関係者の連携した努力を必要としている。その最も重要な点は，透明性の高い説明と同意（インフォームドコンセント）と公平な配分である。この基盤点を担っているのが移植コーディネーターといわれる人たちである。

臓器移植に携わるコーディネーターは脳死臓器移植法に則り，臓器移植ネットワークの本部・支部で活躍されている。一方，組織移植に関してはわが国には法的な取り決めはなく，日本組織移植学会のガイドライン（厚生労働省：医薬食品局『生物由来製品の取り扱い基準』を遵守）に則り行われているが，全国的に一律に行われているとは云い難い状況である。この状況を改善する目的で，日本組織移植学会は「組織移植コーディネーター育成規準」「組織移植バンク開設規準」などを作成しているところであるが，わが国での組織移植ネットワーク作りにおける一連の事業のなかでコーディネーターの育成は最も重要な課題であった。本書は，社会的にも経済的にも厳しい状況におかれているわが国の組織移植医療に，情熱をもって取り組んでこられた二人の専門家により完成したおそらく初めての書である。折しも，西日本でも東日本組織移植ネットワークを見本として西日本組織移植ネットワークを構築中であり，新たに組織移植コーディネーターを育成しているところであるが，日本組織移植学会の監修によりコーディネーターのための教科書となる本書が出版されたことは極めて重要である。

本書は移植医療に携わり，支えている広い領域の医師，看護師，臨床工学士，臨床検査技師，そして臓器・組織移植コーディネーターの座右の書として御推薦申し上げたく思っている。

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Novel PVA–DNA nanoparticles prepared by ultra high pressure technology for gene delivery

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Abstract

Polyvinyl alcohol (PVA)–DNA nanoparticles have been developed by ultra high pressure (UHP) technology. Mixture solutions of DNA and PVA having various molecular weights (Mw) and degree of saponifications (DS) were treated under 10,000 atmospheres (981 MPa) condition at 40 °C for 10 min. Agarose gel electrophoresis and scanning electron microscope observation revealed that the PVA–DNA nanoparticles with average diameter of about 200 nm were formed. Using PVA of higher Mw and degree of saponifications, the amount of nanoparticles formed increased. The driving force of nanoparticle formation was the hydrogen bonding between DNA and PVA. In order to apply the PVA–DNA nanoparticles for gene delivery, the cytotoxicity and the cellular uptake of them were investigated using Raw264 cell lines. The cell viability was not influenced whether the presence of the PVA–DNA nanoparticles. Further, the nanoparticles internalized into cells were observed by fluorescent microscope. These results indicates that the PVA–DNA nanoparticles prepared by UHP technology showed be useful as drug carrier, especially for gene delivery.

Keywords: Ultra high pressure; Hydrogen bond; Nanoparticles; Biocompatibility; Gene delivery; Polyvinyl alcohol

1. Introduction

Pressure processing technology has been used in many fields. The range of pressure is varied in each method from 1 to 100,000 atmosphere (atm) (9810 MPa). In the field of chemistry and biology, the pressure of over 6000 atm is thought as ultra high pressure (UHP). It is well known that the hydrogen bond is strengthens than electrostatic and hydrophobic interactions under the UHP condition [1–3]. From this fact, we recently reported that UHP is one of powerful tools for manipulatory inter- or intra-molecular interaction triggered by hydrogen bond [4]. We have shown some evidence of this hypothesis by using polyvinyl alcohol (PVA), which is synthetic hydrogen bonding polymers having simple hydrogen bonding structure, associated each other to form nanoparticles via hydrogen bond by UHP processing [5]. Among various fields of application, we focused on the usage of the nanoparticle as drug and gene delivery system.

Nanoparticles as gene carrier are able to enhance intracellular gene delivery in vitro and in vivo due to protection of DNA from nuclease cleavage [6–10]. Many types of them, such as cationic compounds [6–8], biodegradable polymers

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[9,10] have been developed. Nanoparticles containing DNA have been formed by electrostatic interaction between negative charge of phosphate groups of DNA and positive charge of cationic compounds or encapsulation. However, it was reported that such cationic substances has the essential problem of the cytotoxicity, and the difficulty of controlling of DNA release from nanoparticles.

In the present study, we report the preparation of novel nanoparticles of plasmid DNA and PVA via hydrogen bond using UHP technology and their application for gene delivery. The interaction force of nanoparticle formation is hydrogen bond between PVA and DNA, because DNA is one of typical hydrogen bonding polymer as well as PVA. Further, the biocompatibility and neutral charge nature of PVA allows the low cytotoxicity. The cellular uptake of them was investigated in order to evaluate the nanoparticles as biocompatible gene carriers.

2. Materials and methods

2.1. Preparation of PVA–DNA nanoparticles by UHP method

PVAs having different molecular weights and degree of saponifications were supplied from Kuraray (Osaka, Japan) (Table 1). Plasmid DNA encoding green fluorescent protein under cytomegalovirus promoter (pEGFP-C1) was obtained from BD Biosciences Clontech (Tokyo, Japan). PVA solutions (0.0001–0.1 w/v%) and pEGFP-C1 solution (0.02 w/v%) were mixed in water and treated under 10,000 atm at 40 °C for 10 min (UHP method) using high-pressure machine (Dr. Chef, Kobe Steel, Kobe, Japan).

2.2. Characteristics of PVA–DNA nanoparticles

At 0.0001–0.01 w/v% of PVA concentration, PVA and pEGFP-C1 mixture solutions treated with UHP were analyzed by agarose gel electrophoresis (1.0 w/v%, 100 V, 1 h). At 0.025–0.1 w/v% of PVA concentration, after centrifugation of the UHP-treated mixture solutions at 5000 rpm for 5 min, the supernatant was collected and the precipitation was washed by water. This procedure was carried out twice. The precipitation was melted by heat treatment for 10 min. They were electrophoresed though 1.0 w/v% agarose gels at 100 V for 1 h. The gels were stained with ethidium bromide. The shape and size of structures were observed by scanning electron microscope (JSM-6301F, JEOL, Tokyo, Japan).

Table 1
Various polyvinyl alcohols used

PVA	DP[a]	DS[b]	Mw
PVA205	500	88	22,000
PVA105	500	98.5	22,000
PVA117	1700	99.3	74,800
PVA140	4000	99.8	176,000

[a] Degree of polymerization.
[b] Degree of saponification.

2.3. Cytotoxicity of PVA–DNA nanoparticles

Mouse macrophage cell lines of Raw264 cells were cultured in a complete modified eagle medium (DMEM, Invitrogen, Tokyo, Japan), supplemented with non-inactivated 10% fetal calf serum (FCS), 50 IU/ml of penicillin, 50 μg/ml of streptomycin (ICN Biomaterials, Ohio, USA). To evaluate the cytotoxicity of PVA–DNA nanoparticles, 2.0×10^4 cells incubated with PVA–DNA nanoparticles at 37 °C for 20 h in the present of FCS and the number of viable cells was assessed using a Cell Counting Kit-8 (Dojindo Laboratory, Tokyo, Japan) according to the manufacturer's instruction.

2.4. Cellular uptake of PVA–DNA nanoparticles

To investigate the cellular uptake of PVA–DNA nanoparticles, pEGFP-C1 labeled with rhodamine by Label It kit (Panvera, WI, USA) was added on 2.5×10^5 cells of Raw264 cells cultured in the present of non-inactivated FCS and incubated at 37 °C for 20 h. The cells were observed under fluorescent microscope.

3. Results and discussion

Fig. 1 shows the microscopic observation of the mixture solutions of pEGFP-C1 and various PVAs at 0.1 w/v% concentration treated with UHP after centrifugation at 5000 rpm for 5 min. The mixture solution of PVA205 remained as clear solution as well as pEGFP-C1. However, a little precipitation was observed in PVA105 and the white precipitation was observed in the case of PVA117 and PVA 140 (Fig. 1(A)). When DNA solution mixed with PVA117 at different concentration were pressurized under UHP condition, the amount of white precipitation was decreased with decreasing PVA concentration, and the precipitation was not observed at 0.01 w/v% of PVA117 (Fig. 1(B)). These results indicate that the size of particle obtained varied in each molecular weight and concentration of PVAs used, and that the higher molecular weights of PVA tended to form particles. This phenomena was observed even when the PVA solution without DNA was treated with UHP (data not shown). Fig. 2 shows SEM images of the UHP treated mixture solutions of DNA in the presence of (A) 0.01 w/v% or (B) 0.025% of PVA. Nanoparticles having average diameter of about 200 nm were observed in 0.01 w/v% of PVA concentration. At 0.025 w/v% concentration, the nanoparticles aggregated each other. It became clear that the precipitation formation at higher PVA concentration under UHP condition due to the aggregation of nanoparticles of PVA or PVA/DNA mixture.