分として存在する。無機材料だから硬くて燃えない性質がある。粉末や板状，時にはスポンジ状(硬い)で用いられる。

ハイドロキシアパタイトには骨の誘導活性があることが知られており，骨の再生には非常に有望だが，いくつか欠点もある。まず，硬くてもろいので成形加工が難しいことがある。細かい骨に加工したり，大きな力のかかる箇所には使いにくい材料だ。また，骨の一成分ではあるが，骨はコラーゲンとハイドロキシアパタイトの複合体である。そのままのハイドロキシアパタイトでは骨の代わりはつとまらない。

そこで，他の成分と複合化させて，生理活性を有しつつ高い強度も併せもつ材料の開発が試みられている。コラーゲン分子の周りにハイドロキシアパタイトの結晶をきちんと並べることができれば，骨と同じ性質をもつ材料が得られると期待されている。一方，ハイドロキシアパタイトは，骨だけでなく皮膚などの軟組織にも非常によく接着することが知られている。この性質を利用して，軟組織の足場材料としての応用が研究されている。

4) 生体組織

細胞足場材料は，再生したい組織のかたちに成形して用いなければならない。耳や皮膚，軟骨などは簡単な構造なのでよいのだが，体の中には複雑な構造の組織がたくさんある。これらの組織を人工的に成形するのが困難な場合があり，心臓についている弁(心臓弁)が好例である。心臓では，3枚の葉っぱのような弁が心臓の拍動とともに形を変え，順方向にはスムースに血液を流し，逆方向には3枚がぴったりくっついて血液を戻さない。

この心臓弁のかたちを成形するのは大変難しい作業である。また，人工物で作成した足場材料は，生体よりも硬いものが多く，体に埋め込むのが大変だったり，硬すぎて機能が発揮できないだけでなく，逆に悪影響を与えることもある。技術が進めば優れた材料が開発されて解決されるかもしれないが，現在の患者を救うために，生物の組織をそのまま使う試みが始まっている。

そのままといっても最低限の加工はする。動物(主にブタやウシ)や亡くなった方から提供された組織から，細胞を除去して組織だけにして，これを用いようというものだ。生体組織そのものといってよいようなしなやかさと強さを有しているから，そのまま用いても役立つ。これを再生医療用の細胞足場材料として用いて，脱細胞化したところに，患者からあらかじめ分離しておいた細胞を埋め込んで，再生を行わせる。

こうすることで，再生途中でも機能を失うことがなく，さらにもともとの生体内の環境と非常に近いので，理想的な組織再生が行われるのではないかと期待されている。生体組織はこれまでにも化学処理(組織中のコラーゲン分子をつなぎ合わせる処理)を施されたものが臨床応用されているが，現在研究されているものは，それらよりも優れた成績を上げつつある。

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解説

澤　芳樹(終章)*
巽　英介(2章)*

単純な役割の裏側に研究者の創意工夫がいかに多く詰め込まれているかを感じ取っていただきたいと思います。

人工皮膚は、主として熱傷（やけど）や褥創（じょくそう）の治療に用いられます。皮膚の機能は、水分蒸散の管理と外来病原菌の排除です。最も簡単な人工皮膚はシリコンゴムのシートでした。ただ単純に傷をカバーするという機能しかありません。

現在の人工皮膚は、多種多様なものがあります。先に挙げた機能を有するのはもちろんのこと、傷の治りが早い、広い面積に使える、病原菌をほとんど完全に防御できる、などの優れた機能があります。また、命を助けるためだけなく、より見た目もきれいに直すことができる「再生医学」の技術を取り入れた最新のものもあります。

人工歯根（しこん）は入れ歯に変わる新しい治療法です。入れ歯自体は江戸時代からある非常に優れた咀嚼（そしゃく）補助具ですが、職人さんの技術がものをいうため、個々の人々にフィットさせることがなかなか困難ですし、手入れも面倒です。人工歯根は、新しい歯を埋め込む方法で、現在、広がりつつあります。

これらの材料系の人工臓器に共通するのは、我々のまわりにある身近な材料を精密に加工することによって優れた医療技術として確立されている、ということです。また、構造や代替する機能が単純である、ということも共通しています。しかし、その

歴史的には、初期には工業用材料の応用が試みられました。私たちが日常の生活に用いている材料は、いろいろな添加物あるいは価格を抑えるために精製過程を簡略化したりしていますが、添加物を厳選し、精製を省略することなく非常に精緻に行なうことによって、医療用の材料として応用されています。

たとえば、人工関節のカップに用いられているプラスチックは超高分子量ポリエチレンです。これは、スーパーマーケットの買い物袋などに用いられていたポリ袋と分子構造は同じものです。しかし、人工関節に用いられているポリエチレンは、分子のつながりを極限まで長くして、かつ長さが揃ったものを集めています。さらに不純物を徹底的にのぞいています。このようにしますと、人間の体重を支えてステンレス製のボールにこすられながら10年以上も耐えることができるようになります。

このように最近では、はじめから医療用を目的として分子設計された材料が開発されてきており、数多くの優れた機能が実現されています。最近、高齢者が寝たきりになることが社会問題化しています。介護保険などの社会保障制度も充実してきましたが、本来ならば、寝たきりの患者さんをださないことが本質的な解決法です。これを手助けするのが人工関節、人工骨です。

構造をいろいろ工夫することによって、光を効率よく透過したり、物質を濾しとったり、酸素を透過したりするような機能を与えることもできます。

さて、体の機能が欠損した部分や機能を補うための人工物を人工臓器と定義すれば、骨や関節、歯、皮膚を置き換えるものも立派な人工臓器です。骨、関節や歯では、非常に大きな力がかかります。ですので、これらの用途に用いるにはまず強度が高くなければなりません。そういうわけで、ここで紹介する構造系の人工臓器には主として金属やセラミックスが使われています。他の人工臓器では小さなパーツとして用いられていた金属・セラミックスがここでは主役です。

人工臓器に用いられる材料に必要な性質はなんでしょうか。まず、生体にとって安全であること、すなわち非毒性が絶対に必要です。また、最低限必要な機能、たとえば強度とか透過性を有していることが必要です。その他にはあまり束縛はありません。したがって、これまでに非常に多くの素材が人工臓器用材料として試されてきました。

現在、臨床に用いられている人工材料は、毒性のないことは無論のこと、長期に埋め込まれても生体に有害でないこと、そして発ガン性のないことなど多くのテストをパスして厳選されたものが用いられています。

に摩擦への耐久性のめやすである「硬さ」など、いろいろな指標に基づいて人工臓器をつくる材料が選ばれています。

すなわち大きな負荷のかかる骨、関節や歯などは、人工心臓や人工血管などと異なって、負荷に耐えること自体が「必要な機能」となります。また人工皮膚は、水分の蒸散を防ぎ、細菌感染をストップする必要がありますが、形は非常にシンプルなシート状をしていて必要な機能のほとんどを材料自身が担当しています。このような材料の特性に依存した人工臓器として、臨床に応用されている人工皮膚、人工歯根、人工関節、人工骨を取り上げました。これらは機能でまとめると、「構造系の人工臓器」とも言えます。

前章までに多くの材料がいろいろな場面で人工臓器に用いられていることをおわかりいただけたと思います。ここで簡単にまとめてみますと、材料としてはプラスチックが大部分を占めています。これはプラスチックが軽い割に強く、加工が容易で安価であるためです。種類もソフトコンタクトレンズのように非常にしなやかで水ともなじみやすいものから、人工心臓に使われているセグメント化ポリウレタンのように、毎分60回の拍動にも耐える高い強度を持つものまで、幅広く揃っています。また分子

解説

構造系人工臓器とは……

構造系人工臓器とは...

人工臓器の多くは人工材料でできているのに「材料系の人工臓器」とは何を指すのでしょうか。

ここでは主に材料自体の特性に依存した人工臓器のことを〝材料系〟としてまとめました。材料自体の特性とは、硬さ、強さ、のびやすさなどを指します。たとえば硬い材料であるガラスやダイヤモンドは、衝撃を加えると簡単に砕けてしまいます。一方、スーパーの買い物袋はプラスチックでぱりぱりしていますが変形は可能です。またかなりの重さの荷物を入れてもちぎれることは滅多にありません。

このように、「硬さ」や「強さ」については、材料の特徴を評価する指標として、数値化して表すことができます。これらは人工臓器の設計に大変重要です。のびたり縮んだりという「変形量（へんけいりょう）」とどれくらいの重さに耐えられるかという「強度」、さら

Early and late stroke after mitral valve replacement with a mechanical prosthesis: Risk factor analysis of a 24-year experience

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Objective: We evaluated risk factors for mortality and stroke after mechanical mitral valve replacement between May 1977 and December 2001.

Methods: Early and late mortality and stroke were assessed. Potential predictors of mortality and stroke were entered into a Cox proportional hazards model. Actuarial survival and freedom from stroke were determined by a log-rank test.

Results: Mitral valve replacement was performed in 812 patients. Concomitant procedures included left atrial appendage closure in 493 (61%) patients, tricuspid annuloplasty-replacement in 348 (43%) patients, maze procedure in 185 (23%) patients, plication of the left atrium in 148 (18%) patients, and other procedures in 151 (19%) patients. Five-year actuarial survival was 91.1% ± 2.3%. Freedom from stroke at 8 years was significantly better in patients with sinus rhythm versus atrial fibrillation ($P < .001$). Ninety-nine percent of patients with mitral valve replacement combined with a maze procedure were free from stroke, whereas only 89% of patients with mitral valve replacement alone were free from stroke at 8 years after surgical intervention. Seventy-two patients had late stroke; sixty-five patients (90%) were in atrial fibrillation, and 47 (65%) patients had the left atrial appendage closed. Multivariate analysis showed that late atrial fibrillation (odds ratio, 3.39; 95% confidence interval, 1.72-6.67; $P = .0001$) and omission of the maze procedure (odds ratio, 3.40; 95% confidence interval, 1.14-10.14; $P = .003$) were the significant risk factors for late stroke.

Conclusions: Persistent atrial fibrillation was the most significant risk factor for late stroke after mechanical mitral valve replacement. Restoration of sinus rhythm with a maze procedure nearly eliminated the risk of late stroke, whereas neither closure of the left atrial appendage nor therapeutic anticoagulation prevented this complication.

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Despite improvements in valve design, stroke remains a serious complication after heart valve replacement. It is generally agreed that lifelong anticoagulant therapy is indicated in all patients with mechanical valves. Recent studies indicate that major systemic embolism still occurs at a rate of 2% to 3% per year after mechanical mitral valve replacement (MVR), despite anticoagulation.[1-3] We and others recently demonstrated that maintaining sinus rhythm by

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Discussion

Dr Frank W. Sellke *(Boston, Mass).* Can you comment on how you performed the biopsies to make sure that there was consistency between the TAA and AAA specimens? Because there is so much, how do I put it, grungy material in aneurysms of both types, especially AAAs, how did you take this into consideration? Also, can you comment on the statistical analysis specific for cDNA array technology?

Dr Absi. Thank you, Dr Sellke. With respect to the biopsies, I obtained TAA specimens from the attending surgeons who were performing the procedure, basically as a ring of tissue removed when they had completed aneurysm repair. This tissue was snap-frozen immediately in liquid nitrogen, and at a later time I extracted the messenger RNA. AAA repair was basically performed by endoaneurysmorrhaphy, so in these cases I obtained a slice of tissue along the whole length of aneurysm itself.

With respect to the statistical analysis, it is true that we did not have a high number of blots. In going over the statistical analysis, especially that provided with the software, our approach was in agreement with literature reports indicating that with 5 blots one can achieve reproducible results. Just because you are dealing with 1185 genes, the parametric analysis must be done with some adjustments in a special kind of analysis of variance, such as the Kruskal-Wallis test, which can be of help when you have a relatively small number of samples.

Dr Michael J. Reardon *(Houston, Tex).* Congratulations on a nice study and a nice presentation. I did have one question about your normal samples. Were they from donors, or were they from the recipients in transplants? Because if they came from donors, they clearly came from much younger people, and there may be altered gene expression with age. We have started taking some plugs from the ascending aorta from coronaries to use those as normal specimens. Would you comment on your normal specimens?

Dr Absi. Thank you, Dr Reardon. I am glad that you brought that up. That is an important issue, and we did not mean to minimize it during the presentation. The pathophysiology of aneurysmal disease is thought to include numerous factors, age and atherosclerosis among them. We chose to use donors for our normal controls, who were young, because we did not want to bias against any of these factors, namely age, in that situation. Age may make a difference and at least some of the results we obtained may be due to age. For this reason we are also planning in the future to compare results in aneurysms with age-matched samples obtained from patients undergoing coronary artery bypass grafting. However, if we had selected or stratified for age in this initial study we would have tended to bias against it, and we did not want to do that here.

Dr Anthony L. Estrera *(Houston, Tex).* This was a nice study. My question is in relation to gender predominance. In our experience in Houston, our patients with thoracoabdominal and ascending arch repairs are primarily male, with a ratio of about 3:2; whereas in our infrarenal replacements there is a greater male predominance, about a 10:1 ratio. Do you have a comment? I appreciate the small numbers in your study, but that would be something to look at in the future.

Dr Absi. We simply chose our patients randomly over a certain period of time during the conduct of the study, without selection for gender, and agree that this will be an important issue to examine in the future.

Dr Larry R. Kaiser *(Philadelphia, Pa).* You mentioned that apolipoprotein E was 14-fold upregulated in, I think, the AAAs. Are you concerned that the high concentration of apolipoprotein E was due to contamination, perhaps by infiltrating foam cells in the arterial wall, and did you use laser capture microdissection to remove some of these foam cells and infiltrating leukocytes that might have interfered?

Dr Absi. No, we did not use that technique, as this study was not designed to identify the cell types or locations where the changes in gene expression occurred, but only to provide a broad survey of changes in gene expression within the tissue samples as a whole. There are clearly a variety of different cell types present within aneurysm tissues, including macrophages and vascular smooth muscle cells, but the cells in which the changes in gene expression were occurring were not examined in this initial investigation. This is another promising approach for the future.

using the Cox maze procedure reduced the incidence of late stroke.[4-6] However, the risk for late stroke after mechanical valve replacement remains to be determined.

The purpose of the study was to elucidate the risks for early and late mortality and stroke after mechanical MVR in a single center over a 24-year period.

Patients and Methods

Between May 1977 and December 2001, we performed 812 MVRs with mechanical prostheses. We retrospectively reviewed the data from operative notes, anesthesia records, clinical case histories, and laboratory investigations, including electrocardiograms, echocardiograms, and cardiac catheterization reports. This retrospective study was approved by the Institutional Review Board of National Cardiovascular Center. Follow-up data were collected from the National Cardiovascular Center records of outpatient visits and correspondence with referring physicians. Each patient was followed by local physicians or by cardiologists or surgeons in our hospital at least every 2 months. A total of 33 clinical, hemodynamic, electrocardiographic, and echocardiographic variables were entered into a computerized database and analyzed. Follow-up data beyond 6 months after the operation were available for all patients.

Definitions

Stroke was defined as cerebral thromboembolism diagnosed by a neurologist and confirmed by means of a computed tomographic scan. Transient ischemic attacks were not counted as strokes in this study. Valve thrombosis was defined as impairment of the valve function by the deposition of thrombus demonstrated at operation or autopsy. Bleeding includes intracranial bleeding, spinal bleeding, and major extracranial bleeding. Intracranial and spinal bleeding were defined as a neurologic deficit of sudden or subacute onset confirmed by means of computed tomographic scanning, surgical investigation, or autopsy. Major extracranial bleeding was defined as an acute bleeding event that led to death or hospital admission or treatment of bleeding; bleeding that led to hospital admission for diagnostic procedures only was not considered major. Bleeding caused by trauma was also excluded. We also excluded all complications that occurred during hospitalization for a different reason because other diseases, interventions, and diagnostic procedures might affect the risk of complications far more than the intensity of the anticoagulant therapy.[7,8]

Patients

Demographic data and preoperative cardiac information are given in Table 1. With respect to preoperative heart rhythm, 78% of patients had chronic atrial fibrillation. In May 1992, the Cox maze procedure was introduced. Between May 1992 and December 1994, the Cox maze III procedure was the procedure primarily performed. A modified maze III procedure (Kosakai maze) was performed until May 1998. The cryo-maze procedure was used thereafter. Details of these procedures have been previously described.[6,9] Five hundred thirty-three (66%) patients had St Jude Medical valves (St Jude Medical, Inc, St Paul, Minn). The remaining patients had a variety of valves including CarboMedics (n = 125; Sulzer Carbomedics, Inc, Austin, Tex), Björk-Shiley (n = 70, in our early experience; Shiley, Inc, Irvine, Calif), ATS (n = 47; ATS Medical, Inc, Minneapolis, Minn), Omniscience (n = 29; Medical Inc, Inver Grove Heights, Minn), and other valves (n = 8). All valves were placed in the antianatomic position.

TABLE 1. Preoperative clinical characteristics

Variable	MVR (n = 812)
Sex (M/F)	331/481
Age (range/median)	18-79 (58)
NYHA (class III/IV) No (%)	482 (60%)
History of stroke No (%)	138 (17%)
Left atrial thrombus No (%)	114 (14%)
Previous cardiac surgery No (%)	369 (45%)
Chronic AF No (%)	630 (78%)

NYHA, New York Heart Association; *AF*, atrial fibrillation.

Assessment of the Intensity of Anticoagulant Therapy

The incidence of adverse events for specific levels of intensity of anticoagulation were calculated as the ratio of the number of events that took place when the prothrombin time was in a particular international normalized ratio (INR) range to the number of patient-years during which the INR was at this level in the patient population. This method has been described in detail previously.[10]

Statistical Methods

Survival and freedom from mortality, atrial fibrillation, and stroke were estimated by using the Kaplan-Meier method. Survivorship curves were compared with a log-rank test. The risks for death, recurrence of atrial fibrillation, and stroke were analyzed by using both univariate analysis and multivariate analysis with a Cox proportional hazards model.

Results

Pathophysiology of Mitral Valve Disease and Operative Findings

The pathophysiology of the mitral valve disease and the nature of concomitant procedures are depicted in Table 2. All patients underwent surgical intervention with mild hypothermia. Antegrade crystalloid cardioplegia was primarily used before 1987, and combined antegrade-retrograde blood cardioplegia was extensively used thereafter. Fifty-one percent (320/627) of the patients undergoing MVR alone underwent concomitant ligation of the left atrial appendage, whereas 94% (173/185) of the patients who underwent combined MVR and the maze procedure had the left atrial appendage closed.

Postoperative Morbidity and Mortality

Hospital death occurred in 33 (4.1%) patients, and causes of deaths are listed in Table 3. Postoperative complications included bleeding (n = 14), renal failure (n = 6), intraaortic balloon pump insertion (n = 4), mediastinitis (n = 3), left ventricular assist device insertion (n = 3), left ventricular rupture (n = 3), and pneumothorax (n=2).

TABLE 2. Pathophisiology for mitral disease and concomitant procedure

Pathophisiology	MVR (n = 812)
MS No. (%)	237 (29%)
MR No. (%)	223 (27%)
MSR No. (%)	169 (21%)
PVF No. (%)	183 (23%)
Concomitant procedure	
Maze procedure No (%)	185 (23%)
LAA closure No. (%)	493 (55%)
LAA plication No. (%)	148 (18%)
Tricuspid valve surgery No (%)	348 (43%)
TAP No. (%)	330 (41%)
TVR No. (%)	18 (2.2%)
CABG No. (%)	25 (3%)
Others No. (%)	126 (16%)

MS, Mitral stenosis; *MR*, mitral regurgitation; *MSR*, mitral stenosis/regurgitation; *PVF*, prosthetic valve failure; *LAA*, left atrial appendage; *TAP*, tricuspid annuloplasty; *TVR*, tricuspid valve replacement; *CABG*, coronary artery bypass grafting.

TABLE 3. Cause of early and late mortality

Early 33 (4.1%)	LOS	18
	MOF	7
	Cerebral infarction	3
	Cerebral bleeding	1
	Others	4
Late 50 (6.2%)	LOS	10
	MOF	7
	Cerebral infarction	8
	Cerebral bleeding	7
	Others	18

LOS, Low-output syndrome; *MOF*, multisystem organ failure.

TABLE 4. Results of univariate and multivariate analysis for late mortality

	Univariate	Multivariate		
Variable	*P* value	*P* value	Hazard ratio	95% CI
Preoperative NYHA class IV	.0090	.0032	3.39	1.50-7.65
Age >65 y	.0059	.0001	2.71	1.50-4.92

CI, Confidence interval; *NYHA*, New York Heart Association.

Survival and Late Mortality

All patients were observed for at least 6 months after their operations. Actuarial 5-year, 10-year, and 15-year survivals were 91.1% ± 1.8%, 88.4% ± 2.3%, and 85.4 ± 3.2%, respectively (Figure 1). There were 50 (6.2%) late deaths. The causes of late deaths are listed in Table 3. Risk factors for late mortality are depicted in Table 4.

Recurrence of Atrial Fibrillation

We chose to analyze recurrence of arrhythmia after the first 30 days because early postoperative atrial fibrillation might be caused by different mechanisms than those of chronic atrial fibrillation. Freedom from atrial fibrillation in the group undergoing MVR alone at 8 years was 25.9% ± 3.2% compared with 71.6% ± 6.3% in the combined MVR and maze group ($P < .0001$, Figure 2). The incidence of stroke was similar between the patients with sinus rhythm after a maze procedure and those in native sinus rhythm (Appendix 1). Moreover, 46 patients with a combined MVR and maze procedure had a recurrence of atrial fibrillation late after surgical intervention.

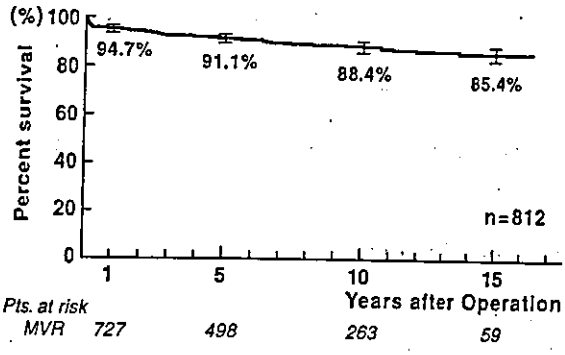

Figure 1. Actuarial survival curve. *Bar* **indicates 95% confidence interval.**

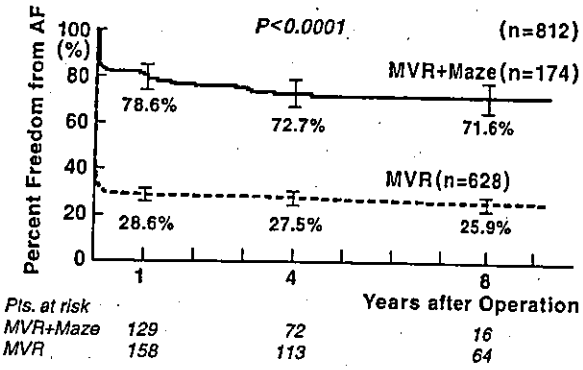

Figure 2. Freedom from recurrence of atrial fibrillation *(AF)*. *Bar* **indicates 95% confidence interval.** *MVR*, **Mitral valve replacement.**

Preoperative and Postoperative Medications

The spectrum and intensity of preoperative antiarrhythmic drugs were similar in patients with and without a concomitant maze procedure. The number of antiarrhythmic agents decreased over time in the combined maze-MVR group, whereas the vast majority of patients undergoing MVR alone continued to receive β-blockers and digoxin for rate control. All patients were taking warfarin, and the target INR was 1.8 to 2.8.[11,12] Only 27 patients took antiplatelet agents; this did not affect the statistical results.

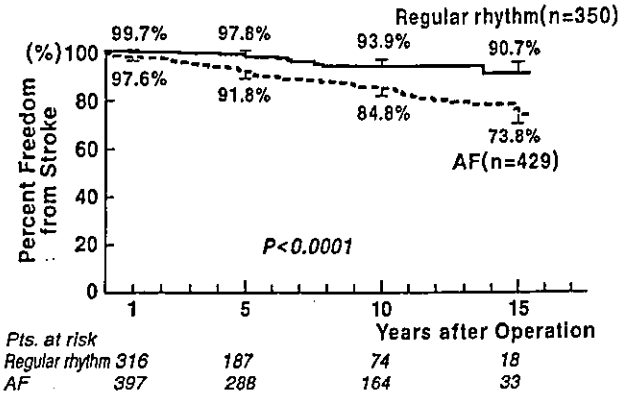

Figure 3. Freedom from stroke stratified by rhythm at 1 month after surgical intervention. *Bar* indicates 95% confidence interval. *AF*, Atrial fibrillation.

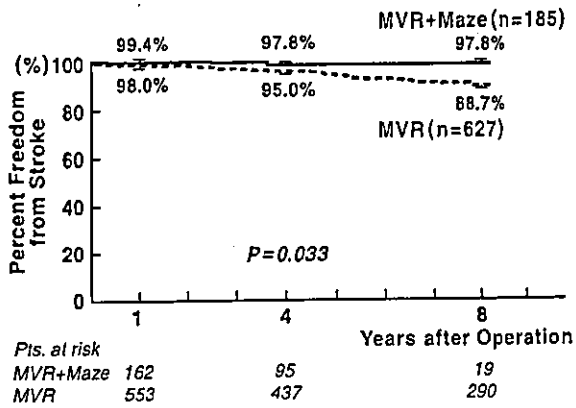

Figure 4. Freedom from stroke: effect of concomitant maze procedure. *Bar* indicates 95% confidence interval.

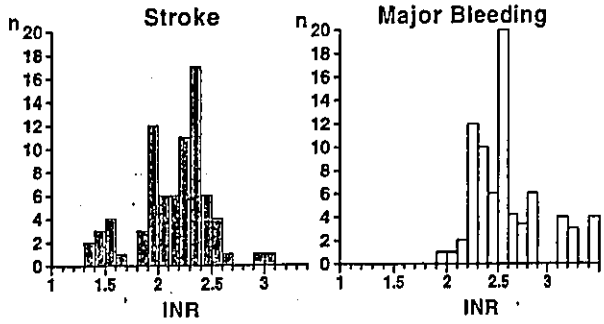

Figure 5. Effect of anticoagulation on stroke and major bleeding after mechanical MVR.

Incidence of Stroke After Surgical Intervention

Seventy-two patients had a late stroke; 65 (90%) patients were in atrial fibrillation, and 47 (65%) patients had the left atrial appendage closed. Irrespective of preoperative rhythm (either in sinus rhythm or atrial fibrillation), closure of the left atrial appendage did not have a significant effect on the incidence of stroke, even in patients undergoing MVR alone. At the time of their stroke, 63 (86%) patients had an INR of greater than 1.8. Nine patients had stopped warfarin for minor operations or dental care; their INR was less than 1.5.

Freedom from stroke 15 years after surgical intervention was 90.7% ± 6.5% in patients with regular rhythm compared with 73.8% ± 6.9% in patients with chronic atrial fibrillation ($P < .0001$, Figure 3). Freedom from stroke 8 years after surgical intervention was 98.7% ± 2.3% in patients who underwent a concomitant maze procedure compared with 88.7% ± 2.6% in patients who underwent MVR alone ($P = .033$, Figure 4). Three (6.5%) of the 46 patients with an unsuccessful maze procedure had a stroke late after surgical intervention.

Incidence of Bleeding After Surgical Intervention

Seventy-six patients had major bleeding episodes after mechanical MVR. Twenty-three of those patients had intracranial bleeding, and the remaining 53 patients had extracranial bleeding. The INR at the time of either stroke or major bleeding is shown in Figure 5.

Risk Factor for Late Stroke

By means of univariate analysis, chronic postoperative atrial fibrillation (at 1 month after surgical intervention), omission of a maze procedure, early year of surgical intervention, and history of stroke were risk factors for late stroke (Table 5). Closure of the left atrial appendage was not a significant risk factor ($P = .69$). By means of multivariate analysis, chronic atrial fibrillation and omission of the maze procedure were the 2 most significant predictors of late stroke (hazard ratio of 3.39 and 3.40, respectively), as determined by using multivariate analysis. Neither closure of the left atrial appendage nor left atrial plication had prevented late stroke.

Discussion

Although MVR with mechanical prostheses is a safe and commonly performed procedure, thromboembolic complications and bleeding related to the anticoagulant therapy remain significant causes of late morbidity and mortality.[13] Recently, we and others reported the effect of the maze procedure on reducing the incidence of late stroke by restoring sinus rhythm. However, the risks for these complications and mortality after mechanical MVR were not fully described.

The current retrospective study confirms that mechanical MVR resulted in excellent 15-year survival (85%, Figure 1). Furthermore, the only risk factors for late mortality were preoperative New York Heart Association class IV and older age (>65 years, Table 4).

TABLE 5. Results of univariate and multivariate analysis for late stroke

	Univariate	Multivariate		
Variable	*P* value	*P* value	Hazard ratio	95% CI
Chronic AF	<.0001	.0004	3.39	1.72-6.67
No Maze procedure	.003	.0279	3.40	1.14-10.14
Year of operation	.013	.0524	1.61	0.99-2.61
History of Stroke	.01	.0003	2.57	1.53-4.32

CI, Confidence interval; *AF*, atrial fibrillation.

This study also confirms that greater than 25% of patients with chronic atrial fibrillation had a stroke during the same 15-year period (Figure 3). Because 78% of the patients who underwent MVR were in atrial fibrillation preoperatively (Table 1), restoration of sinus rhythm after surgical intervention was a critical factor in preventing strokes after mechanical MVR (Figure 3). The use of the maze procedure is a logical strategy to prevent late strokes after MVR with mechanical prostheses.

In the present study a maze procedure combined with MVR resulted in 98.7% freedom from stroke, whereas only 89% of the patients undergoing MVR alone were free from stroke 8 years after surgical intervention.

Moreover, chronic atrial fibrillation and the omission of a maze procedure were the 2 major predictors of late stroke after mechanical MVR. These results indicate that an adjunct maze procedure nearly eliminated the risk of late stroke after MVR with mechanical prostheses.

Among 185 patients who underwent a concomitant maze procedure, 3 patients had a stroke, but all of these patients were in atrial fibrillation at the time of their strokes. Moreover, none had left atrial contraction, which was confirmed by the absence of the a-wave on echocardiography. Patients with a failed maze procedure might have a risk for late stroke similar to that of patients with atrial fibrillation. The radial approach or new radiofrequency techniques preserve a more physiologic atrial transport function.[14,15] Further study is necessary to elucidate the role of left atrial contraction in eliminating left atrial thrombus.[16]

In addition to maintaining sinus rhythm by using a maze procedure, closure of the left atrial appendage or reducing the size of an enlarged left atrium might also be expected to reduce the incidence of late stroke. In this study neither closure of the left atrial appendage nor plication of the left atrium prevented late stroke. However, more than 90% of patients with a combined maze procedure had the left atrial appendage closed, and the cutting and sewing in the original maze procedure could certainly have reduced the size of the left atrium. Differentiation of these factors might be difficult.

This study was also designed to examine the effect of anticoagulation on the incidence of late stroke. Among 72 patients who had a late stroke, 63 had an INR considered therapeutic (1.8-2.8) in the Japanese population (Figure 5).[11,12] Thus therapeutic anticoagulation with warfarin alone is insufficient to prevent late strokes after mechanical MVR, especially in patients with chronic atrial fibrillation. This was a significantly lower target level compared with the American Heart Association/American College of Cardiology guidelines,[7] the recommendations of the American College of Chest Physicians,[17] and several European studies.[18] However, recent studies from Japan indicate that maintaining an INR level between 1.8 and 2.8 results in lower bleeding and stroke rates compared with studies in the United States or Europe.[11,12] Accordingly, the guidelines of the Japanese Circulation Society recommend a target INR of 1.8 to 3.0 for patients after MVR with bileaflet mechanical valves.[19] The overall linearized risk of stroke in this study was only 0.02 per year in patients with atrial fibrillation and 0.004 per year in patients with regular rhythm. Moreover, the majority of patients who had major bleeding had INRs between 2.0 and 3.3 at the time of their events. Thus setting a higher INR might increase bleeding in our population. A prospective randomized study to determine the optimal anticoagulation regimen (eg, warfarin alone vs warfarin plus antiplatelet agents) is necessary.[3,8,18,20]

The major limitation of our study is that it was not randomized. The maze procedure was performed only after 1992, and therefore the follow-up period in the group undergoing MVR alone versus those undergoing MVR plus the maze procedure was different. The follow-up period for the group undergoing MVR plus the maze procedure (mean, 4.45 patient-years; range, 0.5-12.4 patient-years; total, 828 patient years) was significantly shorter compared with that of the group undergoing MVR alone (range, 0.5-22.9 patient-years; total, 5035 patient-years; $P < .0001$). Thus the significantly higher freedom from stroke in the maze procedure group might be related to a shorter observation period. However, our recent study regarding the impact of a maze procedure with mitral valve repair and replacement since 1992 indicated that the addition of a maze procedure significantly reduced the incidence of stroke between the groups during a comparable follow-up period.[6] In addition to this, the decision for or against an adjunctive maze procedure reflected each surgeon's experience. Regarding anticoagulant therapy, the INR was known at the time of a stroke or major bleeding event, but baseline INR was only partly taken into account in this study. Thus the role of compliance as a cause of instability in oral anticoagulant therapy was not fully investigated.[20]

In conclusion, persistent atrial fibrillation was the most significant risk factor for early and late stroke after mechanical MVR. Restoration of sinus rhythm with the maze procedure nearly eliminated the risk of late stroke, whereas neither closure of the left atrial appendage nor therapeutic

anticoagulation prevented this complication. Continuing efforts to define the optimal oral anticoagulant therapy to minimize the chances of stroke and bleeding are still necessary.

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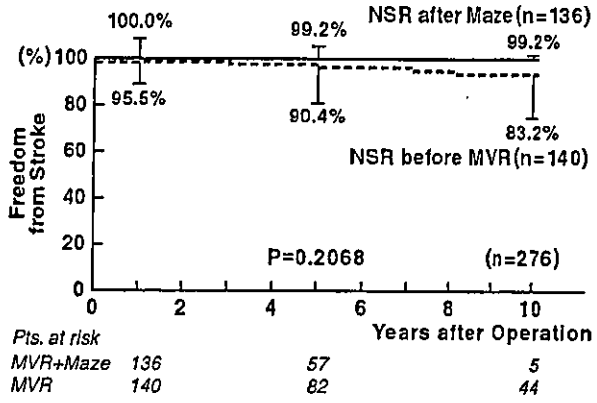

Appendix Figure 1. Freedom from stroke in patients with sinus rhythm after the maze procedure versus freedom from stroke in those in native sinus rhythm.

Discussion

Dr Cary Akins *(Boston, Mass).* I congratulate Dr Bando on this nice presentation of his continuing evaluation of the effect of concomitant maze procedures on the results of mitral valve surgery, which was similar to his presentation at this meeting a year ago. This year's presentation enlarges last year's study with the inclusion of about 40 patients undergoing the combination of the maze procedure and valve replacement and adds about 600 patients who had mechanical MVR from 1977 to 1992, none of whom had a maze procedure. This study concludes, as last year's study did, that sinus rhythm is associated with better long-term neurologic results but that sinus rhythm apparently has no demonstrable effect on long-term survival.

I have several questions about the study design, the first concerning the mechanical prostheses implanted because we know that different prostheses have different thrombogenic potentials. Did all of the patients in this study going back to 1977 receive the same mechanical prosthesis? If different prostheses were used, was prosthesis type inserted as a variable in the Cox analysis?

Dr Bando. Thank you very much, Dr Akins, for your excellent questions. Regarding the type of valves, among these 812 patients, 533 patients had a St Jude mechanical valve, and the remaining valves include the CarboMedics valve in 125 patients and, recently, the ATS valve in 47 patients. Earlier in our experience, the Björk-Shiley valve was used in 70 patients, and the Omniscience valve was used in 23 patients. We did analyze the type of valves as potential predictors in univariate and multivariate analyses, and these were entered into a Cox proportional hazards model, but none of the valve types came out as a risk factor for either late mortality or late stroke.

Dr Akins. My second question focuses on the effect of obliteration of the left atrial appendage on late stroke. The authors include in their statistical analysis of this part of the study patients who had a concomitant maze procedure. Wouldn't it be better to eliminate the maze patients and analyze only patients who had a MVR with or without obliteration of the left atrial appendage?

Dr Bando. That is a very excellent point, Dr Akins. Removing 185 patients, we have 627 patients undergoing MVR alone, and if

you compared those patients with or without closure of the left atrial appendage, actually half of them, 320, have the left atrial appendage closed, whereas the remaining 307 patients had the left atrial appendage left open. Of those, there are 69 strokes we have so far. Again, 43 patients had the left atrial appendage closed, and the remaining 26 were left open. Therefore, looking at those data in this subset of the whole group, there is no difference and no positive effect on the prevention of late stroke.

Dr Akins. In contrast to the program book abstract, the manuscript states that of the 72 patients who had a late stroke, 62 had an INR of only greater than 1.8 and not 2.3. In fact, half of the patients who had a stroke had an INR of 2.2 or less, a level that would be quite low for North American patients with a mechanical mitral valve, particularly those with persistent atrial fibrillation. Have you changed your target INR levels for these patients?

Dr Bando. Actually not, because the second to last slide shows that if the target level is coming out over 3, we are going to have more and more patients with major bleeding, and in our society with the Japanese circulation, we are about to make guidelines of what would be an adequate target level, and it ends up to be between 2 to 3 or 1.8 to 2.8.

Dr Akins. I question using patients going back 25 years as a cohort against which to compare patients having concomitant maze operations since 1992. Earlier patients might have had substantially different operations, prostheses, and other risk factors for stroke. Indeed, earlier year of operation is a significant predictor of late stroke. How do you think the addition of this early surgical group has really added to the strength of your study?

Dr Bando. That is a good point. The observation period is totally different for the maze group studied since 1992 and those patients operated on before that, close to 24 years. Numerous changes have happened in our institution. First, we used the bioprosthetic valve, the Ionescu-Shiley valve, extensively for all generations between 1980 and 1984 and then changed to using the Carpentier-Edwards bioprosthetic valve, but only for patients over 70 years old. Otherwise, we all use mechanical valves. That is the major change. And also, even after 1992, we used 3 different maze techniques. That might have an effect on the results. I agree with you.

Dr Akins. There is no doubt that patients with atrial fibrillation, even those without mitral valve disease, have a greater risk of stroke and are best treated with long-term anticoagulation. Although my bias is to agree with the authors' premise that maintenance of sinus rhythm in addition to anticoagulation for mechanical mitral prostheses might yield better neurologic outcomes, I am not convinced that adding this earlier surgical group has made this study prove that point.

Dr Bando. That is a very good point, and that is the primary reason we are going to do a prospective randomized study to try to find out what would be the best anticoagulation strategy comparing warfarin versus warfarin plus aspirin, and we are going to start that pretty soon.

Direct cell-cell interaction of cardiomyocytes is key for bone marrow stromal cells to go into cardiac lineage in vitro

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Tomita, Fukuhara, Nakatani *(left to right)*

Objectives: Cardiac environmental factors are thought to be powerful inducers in cardiomyogenic differentiation. In this study we simulated the cardiac environment using coculture and evaluated the cardiomyogenic differentiation in bone marrow stromal cells.

Methods: In group 1 only bone marrow stromal cells derived from transgenic mice expressing green fluorescent protein (GFP-BMCs) were cultured (n = 5). In group 2 cardiomyocytes from neonatal rats were grown on inserts, which we applied to culture dishes seeded with GFP-BMCs (n = 5). In group 3 GFP-BMCs were cocultured with cardiomyocytes on the same dishes (n = 5). We cultured these cells for 7 days and evaluated the synchronous contraction and the cardiomyogenic differentiation of GFP-BMCs by means of immunostaining.

Results: In groups 1 and 2 GFP-BMCs protein did not show any myogenic phenotypes for 7 days. In contrast, in group 3 some GFP-BMCs were incorporated in parallel with cardiomyocytes and revealed myotube-like formation on day 1. On day 2, some GFP-BMCs started to contract synchronously with cardiomyocytes. Myosin heavy chain–positive GFP-BMCs were recognized in 2.49% ± 0.87% of the total GFP-BMCs on day 5 ($P < .0001$). Cardiac-specific troponin I–positive GFP-BMCs were in 1.86% ± 0.53% of the total cells on day 5 ($P < .0001$). Atrial natriuretic peptide was also seen in GFP-BMCs, and connexin 43 was detected between GFP-BMCs and cardiomyocytes.

Conclusions: Direct cell-cell interaction with cardiomyocytes was important for bone marrow stromal cells to differentiate into cardiomyocytes. This coculture was useful for simulating the cardiac environment in vitro for the research of cell transplantation in the heart.

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Bone marrow cell may be a candidate for cell-based therapy for regenerating many kinds of tissue such as liver, neuron, fat, and tendon,[1] and we previously reported that transplantation of bone marrow stromal cells (BMCs) induced myogenesis and angiogenesis in damaged hearts and improved impaired function.[2,3]

A part of the transplanted bone marrow cells differentiated into cardiomyocytes without any artificial manipulation in vivo.[2,4-6] Even xenogeneic stem cells went to site-specific differentiation in the body of another species.[7,8] These data suggested that environmental factors were natural inducers of differentiation. The heart might have the capacity to regenerate itself when it is damaged.[9] The effects are very difficult to investigate, however, because of their in vivo nature. We hypothesized that direct attachment between BMCs and cardiomyocytes was one of the environmental inducers.

In this study we simulated the cardiac environment with coculture composed of green fluorescent protein mouse-BMCs (GFP-BMCs) and rat cardiomyocytes and

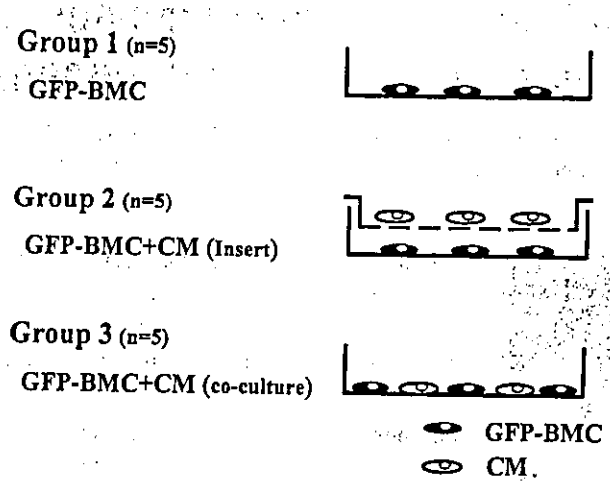

Figure 1. Experimental culture system with rat cardiomyocytes *(CM)* and GFP-BMCs.

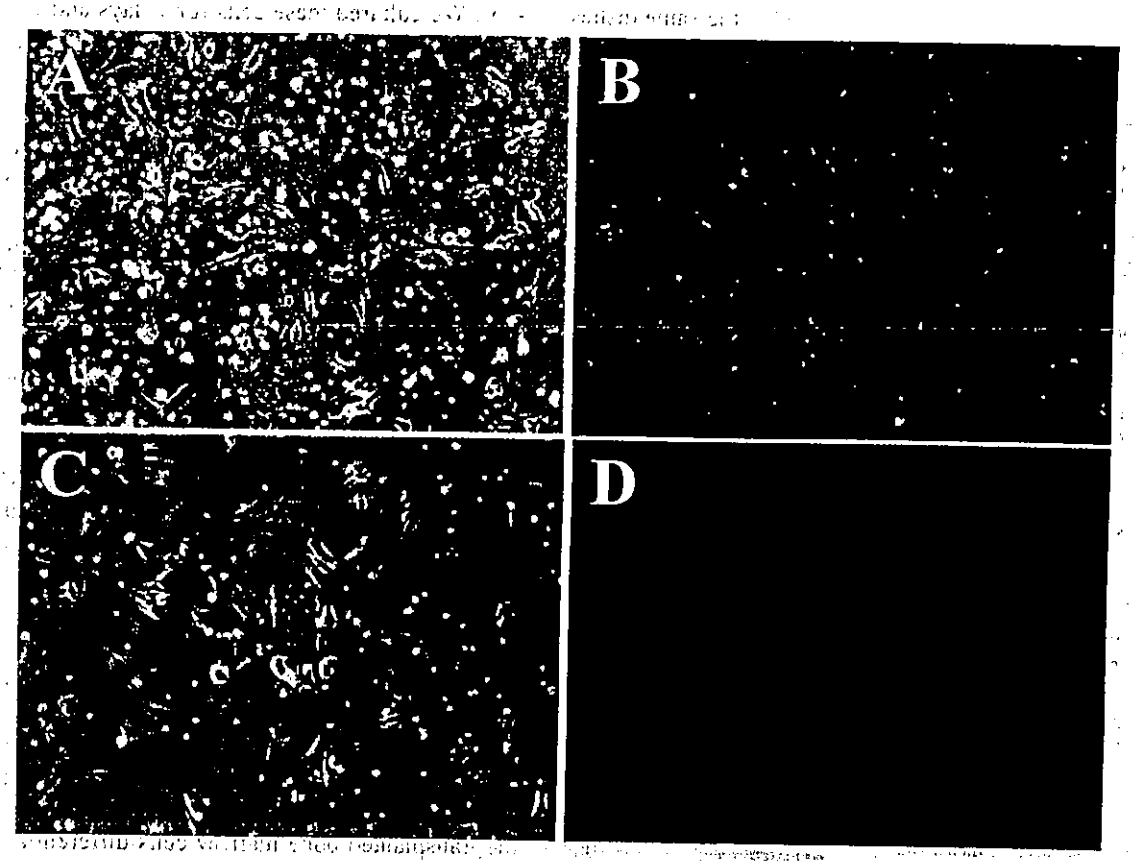

Figure 2. The morphology of GFP-BMCs in passage 2 (A and B) and rat cardiomyocytes (*CM*; C and D) in vitro. A, These cells were spindle, oval, wedge, or sheet shaped. B, All cells expressed green under fluorescent microscopy. (Original magnification 200×.) D, None of the cells were visible under fluorescent microscopy. (Original magnification 200×.)

report, for the first time to our knowledge, that BMCs differentiate into cardiomyocytes in a coculture and cell-cell attachment is one of the environmental factors of differentiation.

Materials and Methods

Subjects

Animals were studied on the basis of the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Labora-

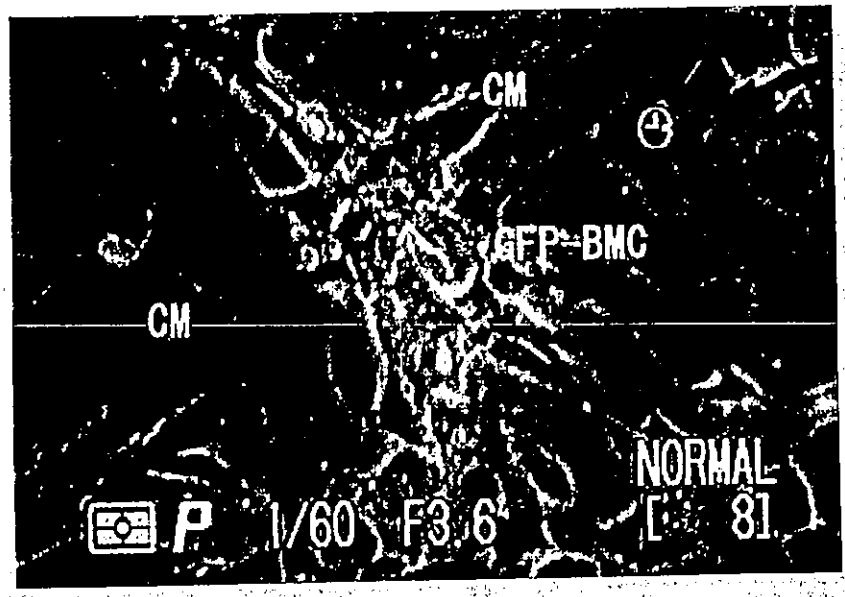

Figure 3. GFP-BMCs with rat cardiomyocytes *(CM)* on day 2 after coculture in group 3. GFP-BMCs were spindle shaped, attached to cardiomyocytes, and contracted synchronously with cardiomyocytes. (Original magnification 200×.)

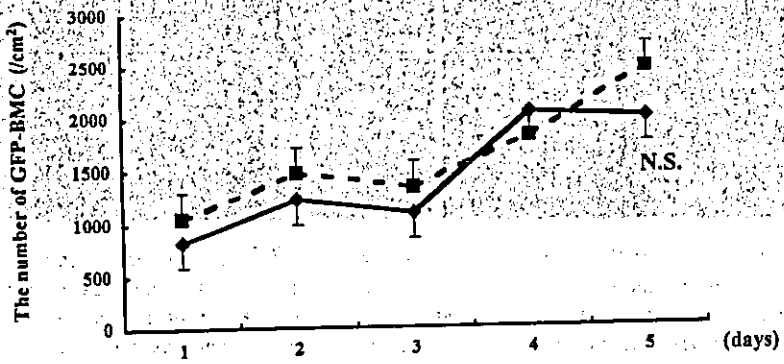

Figure 4. The proliferation of GFP-BMCs in groups 1 *(solid line)* and 3 *(dotted line)* is shown. There was no difference between groups 1 and 3 *(NS)*.

tory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996, and approved by the Institutional Animal Care and Use Committee at the National Cardiovascular Center Research Institute. Pregnant Sprague-Dawley rats were purchased from a licensed vendor. Transgenic mice expressing green fluorescent protein (C57BL/6Tg14[act-EGFP]OsbY01: GFP mouse) were kindly provided by Dr M. Okabe.[10] Animals were housed in an air-conditioned room, with free access to food and water at all times.

BMCs From GFP Mice

A GFP mouse was anesthetized with diethylethanol. After achievement of general anesthesia, the femora and tibiae were collected.[4,11] After removing connective tissue around the bone, both ends of the bone were cut. Bone marrow plugs were flushed with a 27-gauge needle and a syringe filled with complete medium (Iscove modified Dulbecco medium with 10% fetal bovine serum, 100 U/mL penicillin G, and 100 μg/mL streptomycin). Cells were introduced into 100-mm dishes and incubated at 37°C in 5% carbon dioxide and 95% air. Three days later, the medium was changed, and the nonadherent cells were discarded. Medium was completely replaced every 3 days. Passage was done when confluency exceeded 70%. BMCs in passages 2 or 3 were used in this study. We operationally called these cells stromal cells.

Neonatal Rat Cardiomyocytes

Cardiomyocytes were isolated from 1-day-old newborn Sprague-Dawley rats.[12] In brief, neonatal rats were anesthetized with diethylethanol and killed by means of decapitation, and their hearts were rapidly removed and placed into dishes on ice. After the atria