POSTER SESSION #18—PERFUSION AND CIRCULATORY ASSISTANCE TECHNIQUES

Improved Bioresorbable Microporous Intravascular Stents for Gene Therapy

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Drug imbibing microporous stents are under development at a number of centers to enhance healing of the arterial wall after balloon coronary angioplasty procedures. The authors improved the mechanical strength and reservoir properties of a biodegradable microporous stent reported to this Society in 1994. A combined tubular/helical coil stent is readily fabricated by flotation/precipitation and casting/winding techniques. A two stage solvent swelling technique allows precise adjustment of the surface hydrophilic/hydrophobic balance. These developments permit seven-fold improvement in drug capacity without significantly altering mechanical properties. Stents modified in this manner retain tensile and compressive strength and are suitable for remote deployment. Elution kinetics of these modified stents suggest they are suitable for gene delivery. Successful gene transfer and transmural expression have been demonstrated after implantation of stents impregnated with a recombinant adenovirus carrying a nuclear localizing β-galactosidase reporter gene into rabbit carotid arteries. These studies suggest that surface modified, bioresorbable polymer stents ultimately may be useful adjunctive devices for gene transfer during percutaneous transluminal revascularization. *ASAIO Journal* 1996;42:M823–M827.

In many clinical situations, placement of an intraluminal coronary stent provides an effective adjunct to transluminal revascularization by opposing elastic recoil, improving luminal diameter after dilation and reducing acute vascular occlusion in the setting of flow-limiting dissections. Deployment of current metallic stents is successful in more than 90% of treated vessels,[1] but stented vessels remain subject to subacute thrombosis, with reported rates of 3–20% despite aggressive anticoagulation.[2,3] In addition, permanent metallic endovascular prostheses raise theoretical concerns with regard to flexibility mismatch between the stent and the compliant vessel wall, with the potential for chronic vessel wall trauma that may predispose the vessel to medial atrophy, aneurysm formation, or late reactive hyperplasia.[4]

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Several strategies to overcome the current limitations of transluminal revascularization are under active investigation, including local administration of drugs[5] or genes[6] intended to formally alter the biology of the vessel wall. Efforts to combine these approaches with stent technology, for example the development of heparin coated metallic stents, have been reported.[7]

Theoretically, stents manufactured from biodegradable polymers could provide temporary mechanical support for the vessel wall from the time of transluminal revascularization until remodeling processes have stabilized the instrumented vessel.[8] Subsequent bioresorption of the polymer might eliminate the potential for deleterious long-term effects. We have described the fabrication of biodegradable microporous polymer stents that may have potential advantages compared with these endovascular prostheses,[9] providing a reservoir for local delivery of therapeutic molecules or particles to the vessel wall. We describe modified fabrication procedures that yield improved microporous endovascular stents and also report experiment results demonstrating that surgical implantation of polymer stents impregnated with recombinant adenovirus into rabbit carotid arteries results in efficient gene transfer and expression of a reporter gene in the vessel wall.

Methods

Fabrication and Surface Modification

Tubular microporous biodegradable stents composed of 75:25 poly-L-lactic acid (PLLA, MW = 200,000 D) and poly-ε-caprolactone (PCL, MW = 30,000 D) were fabricated by a modification of the flotation-precipitation procedure described previously.[10] Helical and double-helical stents were fabricated from the same materials (75:25 PLLA:PCL) by a casting/winding technique. The polymers were dissolved in chloroform to form a 5% (weight/volume) solution. Polymer films were produced by solution casting, in which approximately 4 ml of the polymer solution was applied to a Teflon coated metal plate and spread uniformly to a rectangular area of 1.5 × 12 cm. After the film was dried for 10 min, it was rolled from one edge to form a fiber. The resulting fibers were wound onto a mandrel to form a helical configuration, annealed at 130°C for 45 min, and the helical stents slipped from the mandrel after swelling in 50% ethanol for 2 hr. Compound stents, in which tubular layers of polymer were

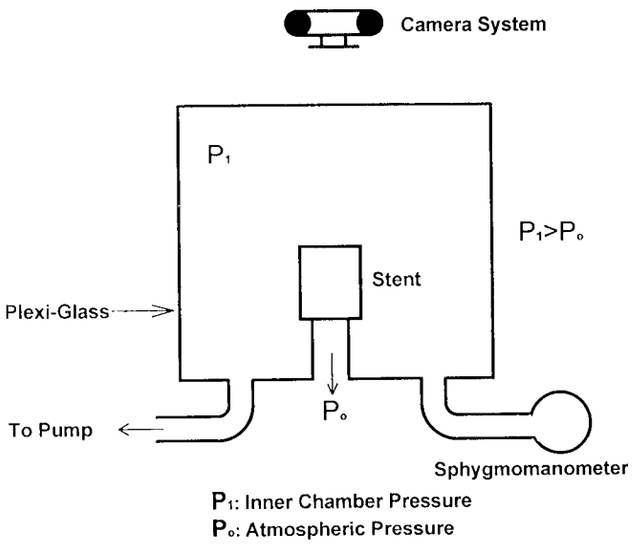

Figure 1. Radial compression test chamber schematic.

cast over a helical skeleton, were produced by employing mandrels carrying helical structures in the flotation-precipitation procedure. The stents were modified by a two stage process. Poly (ethylene oxide) (PEO; MW 18,500 D) was dissolved in deionized water to a final concentration of 0.4 g/ml, and the aqueous PEO solution was added to acetone to produce a 65% acetone/35% aqueous PEO mixture (final PEO concentration approximately 14% w/v). The stents were immersed in this solution for 90 min at ambient temperature with periodic agitation, washed with a large quantity of deionized water, and dried at 60°C for 1 hr. After the stents were dried, they were immersed in a 50% tetrahydrofuran (THF, Aldrich)/50% aqueous PEO mixture (final PEO concentration approximately 8% w/v) at 60°C for 90 min. The stents were washed in a large quantity of deionized water and dried at 60°C for 1 hr. For some experiments, stents were sterilized by one of two techniques, autoclaving (30 min dry cycle) or gas (1 hr ethylene oxide).

Bulk and Surface Structural Analysis

Stents were fixed in liquid nitrogen; circular cross-sections were cut, mounted on aluminum stubs, sputter coated with gold-palladium, and imaged using a JEOL 840-A (Chicago, IL) scanning electron microscope at 15 KeV, 35–39 mm working distance, and 70–400 × magnification. Two-dimensional porosity was estimated by planimetry of electron micrographs, using NIH Image 1.55 software, and expressed as the fractional pore area (pore area/area of the stent wall in cross-section).

Flat strips (4 × 15 mm) were cut from unmodified and modified tubular stents mounted with rubber tipped jaws and subjected to loading at a strain rate of 10 mm/min (Model 1000; Instron, Canton, MA). A chamber was constructed to perform radial compressive tests of the polymeric stents **(Figure 1).** Polymeric stents were mounted inside the glass chamber; a thin plastic sheet was used to prevent air from passing through the microporous stents. A video camera with magnification recorded the change in stent diameter with chamber pressure. Effective radial stiffness, the slope of the stress-strain curve, was calculated from these data.

Water and Virus Uptake and Elution

Wettability of the polymer stents was assessed by determination of water contact angle using the sessile droplet technique, using contact angle goniometry (Model 100-00-115, Ramé-Hart, Inc., New York, NY). Time dependent water uptake was determined by sample immersion, removal of excess surface water by blotting, and weighing.

Tubular polymer stent segments were incubated in virus stock for 1 hr, washed for varying periods of time in Dulbecco's modified eagle medium (DMEM) before adding the segments to CV-1 cells cultured in 10 ml DMEM/10% fetal bovine serum. After addition of the stent segments to the culture medium, cells were reincubated for 48 hr at 37°C and the monolayer harvested for assessment of reporter gene expression by luciferase assay. Each sample was run in duplicate and diluted as necessary to yield luciferase activities within the linear range of the assay. Luciferase activity (light units) for each sample was determined by correcting for background activity and sample dilution. The effective volume of virus stock delivered capacity, and the elution kinetics of the polymer were determined.

Staining for β-Galactosidase Activity

Modified stents were surgically implanted in rabbit carotid arteries. Before being implanted, stents were immersed for 15 min in an isotonic saline solution with $\times 10^{10}$ plaque forming units (pfu)/ml AdCMβGal. The stents were manipulated into approximation with the vessel wall using microfine surgical instruments. After 4 days, the instrumented arteries were isolated, harvested, and submersed in X-gal staining solution for 16 hr at room temperature. Sections were lightly counterstained with eosin and examined for the presence and location of nuclear localizing β-galactosidase activity, as indicated by blue staining cell nuclei.

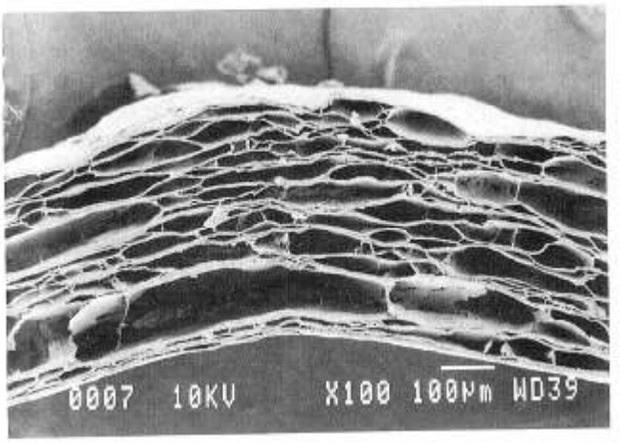

Figure 2. Scanning electrom microscopic examination of typical cross-section of modified stent.

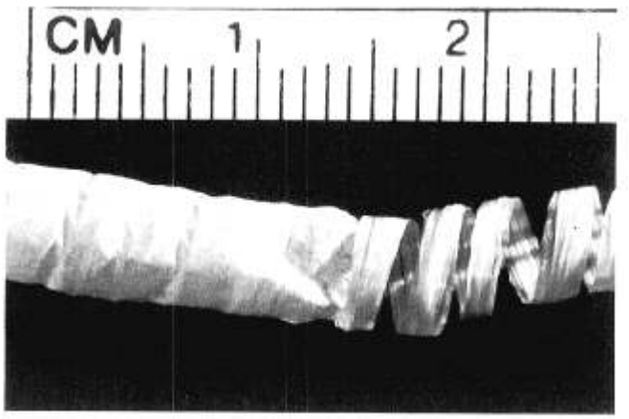

Figure 3. Cutaway view of microporous tubular stent with flat helix reinforcement.

Results

As previously reported, tubular 75:25 PLLA:PCL stents fabricated by the flotation-precipitation technique demonstrate significant porosity. In addition, by altering the polymer solution feeding rate during the fabrication of a single device, a gradient in porosity from the luminal to abluminal surfaces of the stent could be produced. Stents subjected to the two stage surface modification procedure demonstrated small but reproducible changes in porosity (typical cross-section of modified stent) **(Figure 2).** Modified stents demonstrated slightly greater porosity, particularly an increase in pore size in proximity to the outer wall in comparison to unmodified stents. The modification process increased the quantitative porosity of identically fabricated stents from 40 ± 2% to 48 ± 1% in one series of samples. The fractional porosity of the stent wall, determined by planimetry of scanning electron micrographs, ranged from 40 to 50%.

Tubular stents manufactured without reinforcement have little resistance to radial compression. Thus, compound structures incorporating helical or double-helical skeletons were produced. The cutaway view of such a compound coil/tube stent is shown in **Figure 3.** Scanning electron microscopic study of cross-sections of the resulting stents demonstrated that the nonporous helical fiber skeleton was integrally incorporated into the tubular wall, which retained its porous character **(Figure 4).**

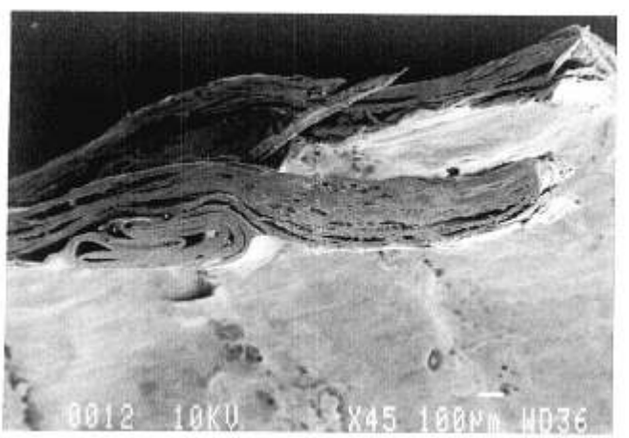

Figure 4. Scanning electrom microscopic examination of wall cross-section of compound stent.

Table 1. Mechanical Properties of Stents

	Tensile Strength	
	Tensile Strength (Mpa)	Young's Modulus (Mpa)
Unmodified porous stent (circumferential)	9.7 ± 3.7	120.9 ± 11.5
PEO modified porous stent (circumferential)	9.0 ± 3.1*	128.0 ± 22.7†
PEO modified porous stent (axial)	5.6 ± 3.0	221.0 ± 89.1
	Compression strength	
	Strength (mmHg)	Stiffness (mmHg)
Nonreinforced stent	772	166
Reinforced stent	5,581‡	1,039‡

* $0.4 < p < 0.5$.
† $0.025 < p < 0.05$.
‡ $p < 0.001$.

In our previous report, we used a trifluoroacetic acid (TFA/PEO) surface modification to improve hydrophilicity but lost most of the mechanical strength. Thus, a two step surface modification was developed. Axial and radial compressive strengths of modified tubular and compound helical/tubular stents were determined. As shown in **Table 1,** incorporation of a polymer coil fiber skeleton dramatically enhanced the capacity of these stents to resist compressive radial forces by seven- to ten-fold. The ultimate tensile strength and Young's modulus of samples were decreased slightly by the modification procedure. Scanning electron microscopic comparison suggested that neither ethylene oxide gas nor autoclave sterilization significantly altered the bulk structural integrity.

Unmodified stents fabricated from this blend of aliphatic polymers were quite hydrophobic. Advancing and receding contact angles were significantly reduced by the one stage surface modification, and additionally reduced (by 43% and 65%, respectively, in comparison to unmodified material) by the two stage surface modification process **(Table 2).** The hydration capacity and kinetics of water uptake are illustrated in **Figure 5.** As shown, unmodified polymer stents demonstrated little water uptake. Surface modification increased the uptake of water by seven-fold, from approximately 5% to 35–40% of dry mass.

Table 2. Average Water Contact Angles (degrees)

	Advancing	Receding
Control	66.4 ± 1.6	56.2 ± 5.8
First step	56.3 ± 1.5*	46.8 ± 6.5*
Modified	37.8 ± 1.9*	19.5 ± 18.5*

* $p < 0.001$.

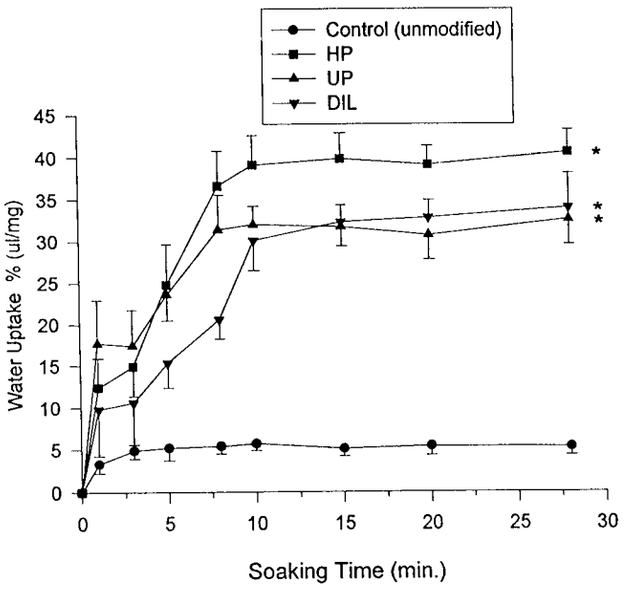

Figure 5. Hydration capacity kinetics of stents. HP-High porosity in the outer surface; UP—Uniform porosity; DIL—Dense inner lumens. HP, UP, and DIE include two step modification. *$p < 0.001$. Error bars: standard deviation.

We also examined the capacity of PEO modified 75:25 PLLA:PCL stent material to take up and deliver recombinant adenovirus *in vitro*. The effective volume of virus stock delivered as a function of sample mass was calculated by comparison of luciferase activity in these cultures 48 hr later against a standard curve. The volumes of virus stock delivered in these experiments is 25–30% of dry mass **(Figure 6),** which was similar to the hydration capacity determination described, averaging more than 80% of the volume predicted from the masses of the stent samples. These results suggest that the surface modified polymer stents both efficiently imbibe virus particles in aqueous medium and elute them in an infectious form **(Figure 7).** An estimated 15% of the absorbed virus would be predicted to remain stent associated for >10 min, a lower estimate of the time required for deployment of such a device *in vivo*.

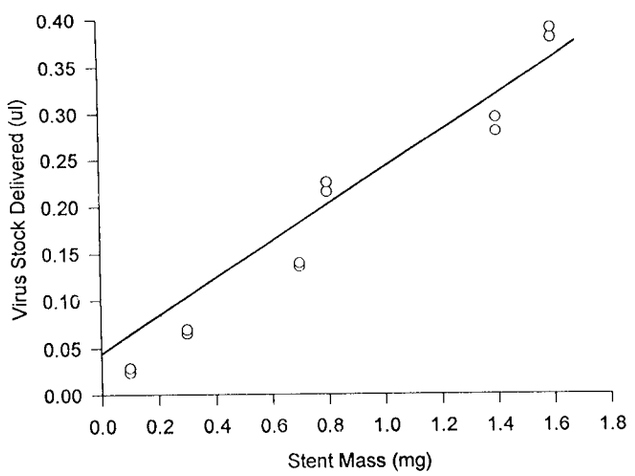

Figure 6. Delivery capacity of modified stents.

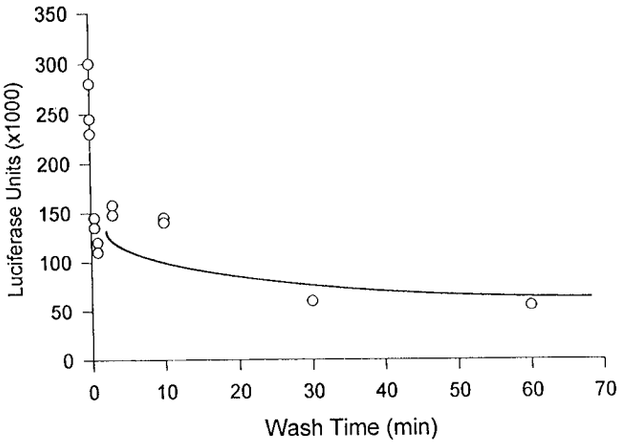

Figure 7. Stent virus elution kinetics.

Surface modified polymer stents impregnated with Ad-CMVβGal were surgically implanted into rabbit carotid arteries. Sections stained for β-galactosidase activity demonstrated expression of the reporter gene focally within the medial layers. Regions of transgene expression corresponded to islands of viable medial smooth muscle cells and, in some cases, cells in the adjacent adventitia.

Discussion

Development of a structurally effective and biologically active resorbable stent hinges on a number of important considerations, including: 1) mechanical properties (strength, recoil stiffness, flexibility, and expansion ratio for remote deployment); 2) gene delivery capacity (hydrophilicity, porosity, and release kinetics); 3) potential toxicity (thrombogenic and inflammatory effects); and 4) biodegradability and the rate at which structural integrity decays. In the current experiments, we evaluated the modified stents in terms of mechanical properties, porosity distribution, hydration capacity, recombinant virus delivery, and gene expression *in vivo*. We demonstrated that surface properties can be adjusted. The surface modification process improved wettability without significant variation in surface morphology or degradation of structural integrity. This is attributed to the two step modification being primarily a solvent swelling (not an acid etching) process, as was the original one step process. The outer surface was swollen by acetone in the first stage, permitting PEO diffusion into and incorporation within the polymer. The use of THF at the second stage increased the rate of hydrolysis and of chain-scission of the PCL component. Thus, more PEO diffused into the loosened polymer structure. The PEO chains are projected from these hydrated surfaces. The incorporated PEO chains coordinate with bound water, thus improving wettability, as indicated by the decreasing contact angle and water uptake studies.

The microporous structure, which averaged 50% porosity after stent modification, provides a higher capacity for drug

uptake. The untreated sample absorbed water at 2–5% of dry weight, whereas the hydration of modified stents was improved to nearly 35% of dry weight, which is in agreement with the theoretical value predicted from the porosity results. This observation indicates that most pores are filled with water and implies that pores are interconnected. The virus stock delivery study yields results that were in agreement with the theoretical delivery predicted from the water uptake study. This confirms that most absorbed virus can be eluted in an infectious form.

The original intent of bioresorbable intravascular stents was to temporarily maintain the lumen of a disrupted artery and ensure continual blood flow after angioplasty. The fabrication and modification process described in this report dramatically increased the hydration capacity, with only minor loss in mechanical strength. Our work indicates that microporous, surface modified stents exhibit adequate strength to permit function as scaffolding devices for diseased blood vessels and also permit delivery of significant quantities of vectored gene solution. The *in vivo* gene delivery experiments demonstrated significant expression of the βGal reporter gene in viable cells of the vessel wall.

Our additional work will focus on an animal model (pig) using these biodegradable microporous devices. Recently, the reinforced stents mounted on balloon delivery catheters have been successfully remotely deployed from carotid artery to femoral artery and renal artery.

Conclusions

A bioresorbable microporous vascular stent can be fabricated and modified by a two step PEO penetration technique to improve water uptake and gene delivery characteristics. Our experiments demonstrate that microporous arterial stent implantation is feasible. Additional work is needed to improve the stent expansion ratio, facilitate remote deployment, optimize the elution properties, and reduce thrombogenicity.

Acknowledgments

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Development of an Integrated Artificial Heart-Lung Device for Long-Term Cardiopulmonary Support

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An integrated artificial heart-lung device has been developed as a long-term cardiopulmonary support system. The device is composed of gas exchange and pumping units. The gas exchange unit consists of a special hollow fiber membrane that can prevent serum leakage. The entire blood contacting surface of the gas exchange unit is treated with covalent heparin bonding. The pumping unit consists of two pusher-plate artificial hearts joined to each end of the artificial lung unit. The core size and priming volume of the device are 11 × 14 × 17 cm and 400 ml, respectively. In *in vitro* evaluation, the device exhibited a maximum output of 7.0 L/min, with a pressure gradient of 10 mmHg per 1 L/min flow rate. In acute *in vivo* evaluation with adult goats, the device satisfactorily replaced the animals' circulation and respira-