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Fibrin-Film Stenting in a Porcine Coronary Injury Model: Efficacy and Safety Compared With Uncoated Stents

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Objectives. **This study was designed to test the efficacy and safety of a fibrin-film–covered stent compared with that of a bare metal stent in the porcine coronary injury model.**

Background. **Biodegradable stents are a potential method of achieving total lesion coverage and delivering local, lesion-specific drug therapy.**

Methods. **Two coronary arteries in each pig were randomly assigned to deployment of either a fibrin-film or a bare tantalum wire-coil stent. An oversized balloon injury, 1.15 to 1.30 times the reference vessel diameter, was induced in each coronary segment before stenting to simulate angioplasty injury. Thirty pigs were studied: group 1 for 28 days (15 pigs); group 2 for 90 days (5 pigs); group 3 for 6 months (5 pigs); group 4 for 1 year (5 pigs).**

Results. **Two pigs died of occlusion of the bare stent and one of occlusion of the fibrin stent ($p > 0.99$). There were no significant differences between the fibrin-stented and bare-stented coronary segments with regard to arterial injury. In group 1 (28 days, 14 pigs), the mean neointimal thicknesses in the fibrin-stented and bare-stented groups were 0.57 ± 0.31 and 0.57 ± 0.27 mm, respectively ($p = 0.89$). In groups 2 to 4 (90 days, four pigs; 6 months, four pigs; 1 year, five pigs), the mean neointimal thicknesses for fibrin- and bare-stented coronary segments at the times studied were 0.48 ± 0.26 versus 0.50 ± 0.22 mm at 90 days; 0.35 ± 0.04 versus 0.35 ± 0.16 mm at 6 months; and 0.33 ± 0.14 versus 0.30 ± 0.14 mm at 1 year ($p = 0.98$).**

Conclusions. **Fibrin-film stents appear to be an excellent candidate for local drug delivery because they can completely and safely cover the stented coronary segment while degrading slowly over 1 to 3 months. This result is important when compared with the poor results of previous studies of synthetic polymer stents.**

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Intracoronary stents have been shown (1,2) to reduce the incidence of restenosis compared with balloon angioplasty in randomized trials of specific patient groups. The reduction in restenosis achieved with these devices results from greater initial lumen gain, prevention of elastic recoil and attenuation of the arterial remodeling process (3,4). Metal stents by themselves do not inhibit the neointimal response to coronary injury but actually stimulate this process, with histologic studies in pigs (5,6) and intravascular ultrasound evaluation in patients (7,8) identifying neointimal hyperplasia as the cause of in-stent restenosis.

Studies using a porcine coronary restenosis model indicate that the structural integrity of the internal elastic lamina is essential to minimize neointimal thickening (5). Exposure of arterial components deep to an injured internal elastic lamina to the blood initiates the first, thrombotic phase of the restenosis process (9,10). A stent capable of covering an entire lesion area uniformly might thus attenuate the neointimal response to vessel injury and reduce the severity of neointimal hyperplasia.

Biodegradable stents are an alternative method of maintaining patency during the restenosis period while achieving total lesion coverage. There is also the potential to deliver local, lesion-specific therapy. A recent multicenter animal study using five biodegradable and three nonbiodegradable polymers incorporated in wire-coil stents, in a porcine model without previous coronary injury, demonstrated a universal marked inflammatory response with neointimal thickening (11). Using the same model, initial results with fibrin-coated stents were more encouraging (12).

The present study was designed to test the efficacy and safety of the fibrin-film stent compared with bare metal stenting, up to 1 year after deployment, in a porcine coronary injury model. The findings may be of considerable interest because the fibrin-film stent can provide complete endoluminal paving by covering 100% of the arterial surface, compared with the partial coverage achieved with the metal stent alone.

Methods

Animals. The study was performed with the approval of the Animal Care and Use Committee of the Mayo Clinic. The

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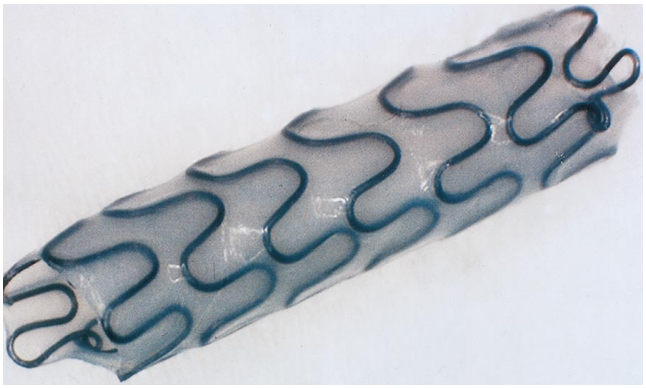

Figure 1. Rehydrated fibrin-film stent.

juvenile domestic, crossbred porcine coronary injury model has been described previously (9). The day before the procedure, pigs were premedicated with aspirin (650 mg), verapamil (120 mg) and ticlopidine (250 mg) orally. Before anesthesia, additional aspirin (325 mg) and ticlopidine (250 mg) were given to each pig. General anesthesia was achieved with intramuscular, followed by intravenous, ketamine (30 mg/kg body weight) and xylazine (3 mg/kg). Additional medication at the time of induction included atropine (1 mg) and flocillin (1 g) intramuscularly. During the stenting procedure, an intraarterial bolus of 10,000 U of heparin was administered. Oral aspirin, 325 mg/day, was continued for the duration of the study. Arterial access was obtained by cutdown on the right external carotid and placement of an 8F sheath. After the procedure, the animals were continued on a normal diet for the remainder of their course.

Stent placement. Two coronary arteries in each pig were randomly assigned to deployment of one fibrin-coated or one bare tantalum wire-coil Wiktor stent. An oversized balloon injury, 1.15 to 1.30 times the reference vessel diameter (based on arterial and nominal balloon sizes), was used in each coronary segment before stenting to simulate angioplasty injury. Segments with major side-branches were deliberately avoided. Both bare and fibrin stents were 15 mm long, hand-crimped on 20-mm balloons and delivered using standard guide catheters and wires. Stents were sized to 1.1 to 1.2 times the reference vessel diameter and the pressure used was uniformly 6 atm for all deployments.

The preparation of the fibrin stents has been previously described (12). Briefly, a fibrin film was prepared from porcine fibrinogen. The fibrinogen was polymerized with porcine thrombin, compressed, dehydrated and gamma sterilized. Just before implantation, the stents were rehydrated for 10 min in 20 to 30 μl of heparin at a concentration of 10,000 U/ml (Fig. 1). Thus, after rehydration and before crimping, there were 200 to 300 U of heparin in the fibrin-film stent. The crimping process is thought to remove roughly half of this amount of heparin, with the remaining heparin washed away by coronary blood flow after deployment.

Morphometric analysis. The pigs were euthanized using a commercial intravenous solution (Sleepaway, 10 ml, Fort Dodge Laboratories). The hearts were then immediately removed and the coronary arteries fixed by pressure perfusion (100 mm Hg) with 10% neutral buffered formalin for 24 h. After fixation, the stented coronary segments were dissected free and cut at 2-mm perpendicular intervals. The tissues were subsequently embedded and stained with hematoxylin-eosin and elastic-van Gieson. Vessel injury severity and neointimal response were measured by calibrated digital microscopy as previously detailed (13). Vessel injury at each stent wire site was scored as follows: 0 = endothelium denuded; 1 = internal elastic lamina lacerated; 2 = media lacerated; 3 = external elastic lamina lacerated. The neointimal thickness was also measured at each wire site, and mean injury scores and neointimal responses were calculated for each stented coronary segment.

Study end points. The end points of the study were to define the efficacy measured by neointimal thickening and the safety of the fibrin-film stent implant compared with that of the bare metal stent. *Death* was defined as procedure related if it occurred within 24 h of stent deployment. *Efficacy* was determined by comparing the morphometric analysis of fibrin-stented and bare-stented coronary segments at 28 days (group 1), 90 days (group 2), 6 months (group 3) and 1 year (group 4).

Statistical analysis. A sample size of 14 arterial segments in each group was required to detect a 25% reduction in neointimal thickness at 28 days, by defining a significance level of 95% (alpha 0.05) and a statistical power of 80% (beta 0.20). Two coronary arteries were treated in each pig; thus, a total of 14 animals were used for 28-day (group 1) analysis. Twelve animals were randomly chosen for extended survival (90 days, 6 months, 1 year) to observe the neointimal response after fibrin-film, compared with bare metal stenting, over these time points (n = 4/group). Thus, allowing for a procedural mortality of 10%, a total of 30 pigs underwent deployment of one fibrin-film and one bare metal stent. Statistics used included the Fisher exact test and two-way analysis of variance.

Results

Sixty stents (one fibrin-film and one bare stent in each pig) were deployed in 30 pigs. The left anterior descending, circumflex and right coronary arteries were each used 10 times for fibrin-film and bare stent deployment. Angiography immediately after the stent procedure demonstrated Thrombolysis in Myocardial Infarction trial (TIMI) grade 3 flow, with no evidence of fibrin sleeve dislodgment, in all cases. A procedure-related death occurred in three animals (10%) that on post mortem was confirmed to be secondary to thrombotic stent occlusion. Two pigs died of occlusion of the bare stent and one of occlusion of the fibrin stent. Therefore, the stent occlusion rates were 6.7% (2 of 30) and 3.3% (1 of 30) for the bare stent and fibrin stent groups, respectively ($p > 0.99$). The number of animals in each group surviving to euthansia was 14 in group 1 (28 days); 4 in group 2 (90 days); 4 in group 3 (6 months); and 5 in group 4 (1 year).

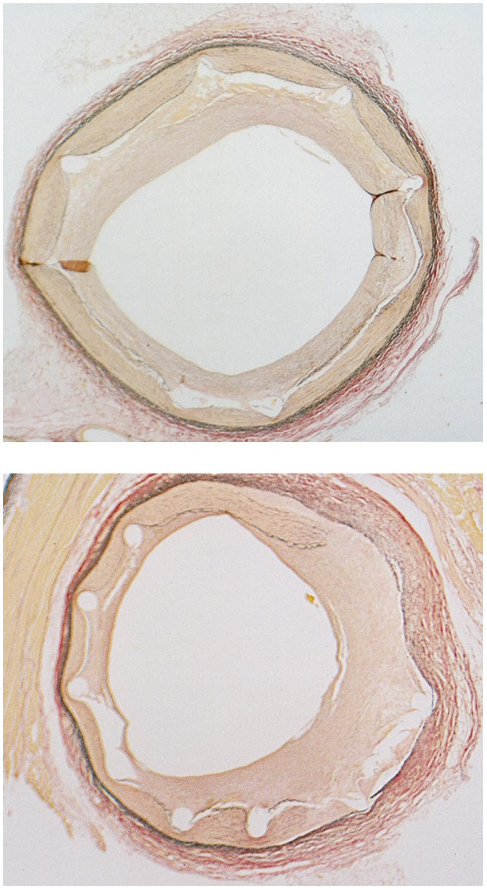

Figure 2. Group 1: representative low magnification sections prepared for morphometric analysis showing a fibrin-film stent with residual fibrin present **(top)** and a bare metal stent **(bottom)** 28 days after deployment.

Group 1. Fourteen pigs were euthanized at 28 days. There were no significant differences between the fibrin-stented (n = 14) and bare-stented (n = 14) coronary segments with regard to arterial injury or neointimal response; the mean neointimal thicknesses in the fibrin- and bare-stented groups were 0.57 ± 0.31 and 0.57 ± 0.27 mm, respectively (p = 0.89). The fibrin stent thus exhibited no more neointimal thickening than the bare stent (Fig. 2) despite achieving complete arterial injury site coverage. This result is reflected by the similar injury-response regression lines for fibrin-film and bare metal stented coronary segments (Fig. 3).

Groups 2 to 4. Four, four and five pigs survived to 90 days, 6 months and 1 year, respectively. The mean injury scores and mean neointimal thicknesses for each of these groups are shown in Table 1. The mean neointimal thicknesses for fibrin- and bare-stented coronary segments at the times studied were 0.48 ± 0.26 versus 0.50 ± 0.22 mm, 90 days; 0.35 ± 0.04 versus 0.35 ± 0.16 mm, 6 months; and 0.33 ± 0.14 versus 0.30 ± 0.14 mm, 1 year. There were no significant differences between the neointimal responses in the fibrin stent and bare stent groups at any of the time points studied (p = 0.98). However, neointimal thickness in both groups decreased over time, from 28 days to 1 year.

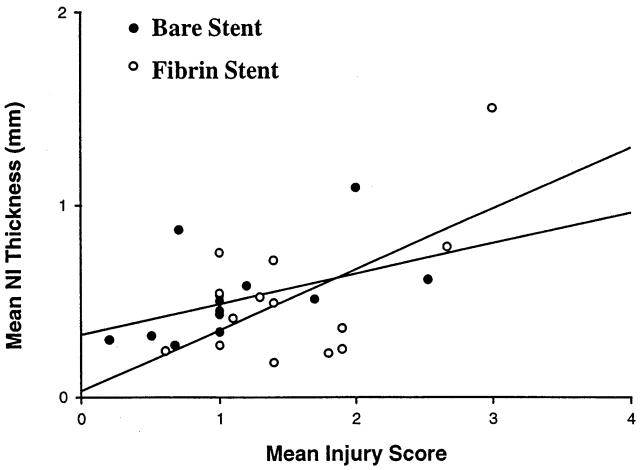

Figure 3. Group 1: injury-response regression analysis for fibrin-film– and bare metal–stented coronary segments.

At 28 days there were only small amounts of exogenous fibrin present, and none was visible at or after 90 days. The arterial segments covered by the fibrin-film stent exhibited no significant foreign body, giant cell or inflammatory reaction up to 1 year after deployment (Fig. 4). The observed neointimal hyperplasia appeared to advance through the gradually degrading fibrin-film.

Discussion

The present study confirms the safety of the fibrin-film stent compared with that of a bare metal stent in a porcine coronary injury model. The fibrin stent thrombosis rate was low (3.3%), given that the animals sustained a significant arterial injury before stenting, and that the pigs received only a single bolus of heparin and no postprocedural ticlopidine. It is possible that rehydrating the fibrin-film stent in a heparinized solution contributed to this low thrombosis rate. The short to midterm data demonstrate no significant neointimal obstruction or late mortality up to 1 year after stenting.

The fibrin-film stent did not inhibit neointimal thickening compared with that for the bare metal stent. The adventitia seems to play an integral role in neointimal response to injury,

Table 1. Groups 2 to 4: Mean (±SD) Neointimal Thickness by Mean Injury Score

Stent	Injury (mm)	Neointima (mm)
Group 2 (n = 4; 90 days)		
Bare	1.7 ± 0.6	0.50 ± 0.22
Fibrin	1.0 ± 0.9	0.48 ± 0.26
Group 3 (n = 4; 6 mo)		
Bare	2.3 ± 0.6	0.35 ± 0.16
Fibrin	2.5 ± 0.2	0.35 ± 0.04
Group 4 (n = 5; 1 yr)		
Bare	2.1 ± 0.3	0.30 ± 0.14
Fibrin	2.1 ± 0.3	0.33 ± 0.14

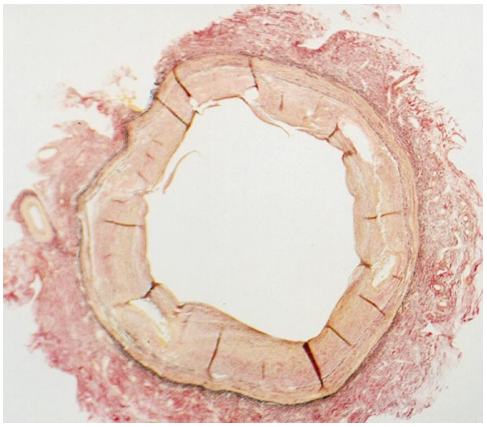

Figure 4. Group 4: representative low magnification section prepared for morphometric analysis showing a fibrin-film stent 1 year after deployment.

which may partly explain the lack of attenuation observed after fibrin-film stenting (13–15). However, this result in itself is important when compared with results of previous synthetic polymer stent studies. In contrast to those studies, the fibrin-film stent did not stimulate neointimal thickening despite achieving total arterial coverage at the treatment site.

Polymer stents. To be clinically applicable, a polymeric stent needs to incorporate sufficient mechanical support to maintain lumen gain and vessel patency. The device should allow endothelialization of the diseased arterial segment after the procedure. In addition, the stent should be biocompatible and should not initiate local thrombus formation or foreign body reaction. Such stents could also act as a vehicle for local drug delivery during the period associated with neointimal thickening.

The porcine model of coronary restenosis, compared with other models in common in use, more closely reflects the process observed in human atherectomy and autopsy specimens (16). Initial results with polymeric stenting using the porcine model were disappointing. A polyethylene terephthalate meshwork stent caused an extensive inflammatory, proliferative response with coronary occlusion in all stented segments (17). In a recent multicenter animal study (11), five biodegradable and three nonbiodegradable polymers incorporated in wire-coil stents were implanted in the porcine coronary model. Five to 10 stents were placed in four to six animals in each of the eight polymer groups. The arterial patency rate at 4 weeks ranged from 70% to 100%. Five polymer groups demonstrated an eccentric coronary lumen reduction angiographically at the site of the stent implant. All eight polymers evoked a marked vessel wall inflammatory reaction. This powerful induction of neointimal proliferation by polymeric stenting would have to be controlled before their potential could be investigated in humans.

Fibrin-film stents. Fibrin has been found to have many promising applications within the medical field, such as enhancing the endothelialization of peripheral vascular grafts in vitro (18) and promoting operative hemostasis during cardiovascular surgery (19–21). Studies in the porcine coronary model indicate that native fibrin is deposited at the site of arterial injury and, in part, determines the neointimal response (5,10). This evidence prompted the first study assessing the response of the arterial wall to fibrin-coated stents (12).

Using the same porcine coronary model, 34 such stents were placed in 20 animals with no acute complications. Follow-up angiography at 28 days confirmed patency in all 31 remaining stented coronary segments. In all cases the fibrin was still structurally intact and completely endothelialized. Importantly, there was no exaggerated neointimal response or foreign body reaction to the fibrin stent, and local vascular integrity was maintained.

The use of autologous vein grafts to achieve total natural stent coverage has been reported with encouraging initial results. In the porcine model, 27 autologous vein graft stents were deployed in iliac arteries, with uncoated stents acting as control stents (22). Follow-up ranged from 7 days to 6 months. All autologous vein graft stents remained patent and became incorporated into the arterial wall after 2 months. Also, no exaggerated neointimal response was observed. The same group has deployed these stents in seven patients without complications for a mean follow-up period of up to 4 months (23).

The present study indicates that the fibrin stent alone does not attenuate the neointimal response to coronary injury compared with that for a bare metal stent. However, fibrin appears to be an ideal candidate for local drug delivery because it can completely and safely cover the stented coronary segment while degrading slowly for 1 to 3 months. This attribute is in contrast to bare metal stents, which cover less than one-third of the lesion surface area on deployment (24) and are thus limited in their ability to deliver site-specific therapy. However, total arterial coverage does make the avoidance of major coronary side-branches a practical concern.

The rate at which fibrin is reabsorbed depends on its formulation. It should therefore be feasible to control the rate of biodegradation by varying the formulation. In the same way, one may vary the matrix size and incorporate microcapsules capable of local drug delivery (25). This approach would add to the potential of the fibrin stent to inhibit the neointimal response that was still observed with the stand-alone device. Commercially available fibrin products were banned by the Food and Drug Administration in 1978 because of concerns about viral transmission (26). The fibrin-film incorporated in the present stent is dehydrated and gamma sterilized before use, thus removing any potential infectious risk. In addition, the fibrinogen used comes from human donors screened for infectious viruses. Alternative sources of fibrin include autologous donation in nonemergent cases (21) and, potentially, transgenic animals or recombinant DNA technology.

Conclusions. In the present porcine model, fibrin acted as an excellent biocompatible and biodegradable polymeric coating when incorporated into metal stents. As such, it has clinical potential as part of a "hybrid stent," composed of a mechanical metal scaffolding with a coating incorporating local drug

delivery, to achieve complete endoluminal paving and deliver antithrombogenic or antiproliferative therapy, or both, uniformly across the arterial injury site. We are presently assessing the capability of the fibrin-film stent to deliver antiproliferative gene and radiation therapy in the porcine restenosis model.

References

1. Serruys PW, de Jaegere P, Kiemeneji F, et al., for the Benestent Study Group. A comparison of balloon-expandable stent implantation with balloon angioplasty in patients with coronary artery disease. N Engl J Med 1994;331: 489–95.
2. Fischman DL, Leon M, Baim DS, et al., for the Stent Restenosis Study Investigators. A randomized comparison of coronary stent placement and balloon angioplasty in the treatment of coronary artery disease. N Engl J Med 1994;331:496–501.
3. Post MJ, Borst C, Kuntz RE. The relative importance of arterial remodeling compared with intimal hyperplasia in lumen renarrowing after balloon angioplasty. Circulation 1994;89:2816–21.
4. Painter JA, Mintz GS, Wong SC, et al. Serial intravascular ultrasound studies fail to show evidence of chronic Palmaz-Schatz stent recoil. Am J Cardiol 1995;75:398–400.
5. Schwartz RS, Huber KC, Murphy JG, et al. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. J Am Coll Cardiol 1992;19:267–74.
6. Karas SP, Gravanis MB, Santoian EC, Robinson KA, Anderberg KA, King SB. Coronary intimal proliferation after balloon injury and stenting in swine: an animal model of restenosis. J Am Coll Cardiol 1992;20:467–74.
7. Hoffmann R, Mintz GS, Dussaillant GR, et al. Patterns and mechanisms of in-stent restenosis: a serial ultrasound study. Circulation 1996;94:1247–54.
8. Mudra H, Regar E, Klauss V, et al. Serial follow-up after optimized ultrasound-guided deployment of Palmaz-Schatz stents. Circulation 1997;95: 363–70.
9. Schwartz RS, Murphy JG, Edwards WD, Camrud AR, Vlietstra RE, Holmes DR. Restenosis after balloon angioplasty: a practical proliferative model in porcine coronary arteries. Circulation 1990;82:2190–200.
10. Schwartz RS, Holmes DR, Topol EJ. The restenosis paradigm revisited: an alternative proposal for cellular mechanisms. J Am Coll Cardiol 1992;20: 1284–93.
11. van der Giessen WJ, Lincoff AM, Schwartz RS, et al. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. Circulation 1996;94:1690–7.
12. Holmes DR, Camrud AR, Jorgenson MA, Edwards WD, Schwartz RS. Polymeric stenting in the porcine coronary artery model: differential outcome of exogenous fibrin sleeves versus polyurethane-coated stents. J Am Coll Cardiol 1994;24:525–31.
13. Schwartz RS, Edwards WD, Bailey KR, Camrud AR, Jorgenson MA, Holmes DR. Differential neointimal response to coronary artery injury in pigs and dogs: implications for restenosis models. Arterioscler Thromb 1994;14:395–400.
14. Barker SGE, Talbert A, Cottam S, Baskerville PA, Martin JF. Arterial intimal hyperplasia after occlusion of the adventitial vasa vasorum in the pig. Arterioscler Thromb 1993;13:70–7.
15. Shi Y, O'Brien JE, Fard A, Mannion JD, Wang D, Zalewski A. Adventitial myofibroblasts contribute to neointimal formation in injured porcine coronary arteries. Circulation 1996;94:1655–64.
16. Schwartz RS. Animal models of human coronary restenosis. In: Topol EJ, editor. Textbook of Interventional Cardiology. Philadelphia: WB Saunders, 1994:365–81.
17. Murphy JG, Schwartz RS, Edwards WD, Camrud AR, Vlietstra RE, Holmes DR. Percutaneous polymeric stents in porcine coronary arteries: initial experience with polyethylene terephthalate stents. Circulation 1992;86: 1596–604.
18. Zilla P, Fasol R, Preiss P, et al. Use of fibrin glue as a substrate for in vitro endothelialization of PTFE vascular grafts. Surgery 1989;105:515–22.
19. Baker JW, Spotnitz WD, Nolan SP. A technique for spray application of fibrin glue during cardiac operations. Ann Thorac Surg 1987;43:564–5.
20. McCarthy PM. Fibrin glue in cardiothoracic surgery. Transfus Med Rev 1993;7:173–9.
21. Tawes RL, Sydorak GR, DuVall TB. Autologous fibrin glue: the last step in operative hemostasis. Am J Surg 1994;168:120–2.
22. Stefanadis C, Toutouzas K, Vlachopoulos C, et al. Stents wrapped in autologous vein: an experimental study. J Am Coll Cardiol 1996;28:1039–46.
23. Stefanadis C, Toutouzas K, Vlachopoulos C, et al. Autologous vein graft-coated stent for treatment of coronary artery disease. Cathet Cardiovasc Diagn 1996;38:159–70.
24. Rogers C, Edelman ER. Endovascular stent design dictates experimental restenosis and thrombosis. Circulation 1995;91:2995–3001.
25. Guzman LA, Labhasetwar V, Song C, et al. Local intraluminal infusion of biodegradable polymeric nanoparticles: a novel approach for prolonged drug delivery after balloon angioplasty. Circulation 1996;94:1441–8.
26. Bove JR. Fibrinogen—is the benefit worth the risk? Transfusion 1978;19: 129–36.