

ANNALS OF  
**Plastic Surgery**

VOLUME 32 NUMBER 6

JUNE 1994

*In this issue:*  
Papers of the Northeastern  
Society of Plastic Surgeons

# Microvascular Anastomoses Utilizing New Intravascular Stents

Martin J. Moskovitz, MD\*

Lawrence Bass, MD†

Ling Zhang, MD†

John W. Siebert, MD†

**Evolving microsurgical technique has allowed for the anastomosis of smaller diameter vessels. Standard suture anastomoses cause a measure of stenosis and intimal damage during application and therefore cannot be dependably used in the anastomosis of very small vessels. We developed and tested a fibrin glue-based anastomosis applied over a meltable stent made of mono- di- and tri-glycerides. In vivo rodent studies using the 0.35 mm diameter inferior epigastric artery have shown immediate and short-term patency rates better than those associated with suture technique. The stent technique is significantly faster and easier than the suture technique. The glyceride stent method suffers from decreased late patency due to aneurysm formation. In addition, we developed a glyceride-coated polyethylene glycol-based stent for use in lasered anastomoses. Work on both projects is ongoing.**

Moskovitz MJ, Bass L, Zhang L, Siebert JW. Microvascular anastomoses utilizing new intravascular stents. *Ann Plast Surg* 1994;32:612-618

From the \*Department of General Surgery, Maimonides Medical Center, Brooklyn, NY, and the †Institute of Reconstructive Plastic Surgery, New York University Medical Center, New York.

Address correspondence to Dr Moskovitz, 180 Clinton St, Brooklyn, NY 11201.

The use of stents in vascular reconstruction is not new. One hundred years ago, Abbe [1] reported the experimental use of permanently placed intraluminal glass tubes in the anastomosis of a canine femoral artery. In 1902, Carrel [2] sought to improve on Abbe's innovation when he used dissolvable intravascular stents composed of caramel candy cylinders. Although these experiments were interesting at the turn of the century, developing suture technology soon remanded these techniques to the pages of history.

In the latter half of this century, however, the diminishing size of anastomosed arteries rekindled the search for an anastomotic method that either supplanted sutures or made their use easier and safer. Most of these efforts were focused on extraluminal cuffing [3-5] or everting ring-pin devices [6]. Routine use of smaller vessels in microvascular surgery and the continuing effort

to improve reconstructive outcomes have brought microsurgery back to the intravascular stent [7, 8] because both suture and coupling device techniques seem to be nearing their technical limitations.

We devised new stented anastomotic techniques for small vessels (range, 0.3-0.8 mm external diameter). We believe these methods may offer a new avenue for microanastomoses.

## Materials and Methods

This experiment began with a materials search to find a biocompatible stent material that would remain solid in the intravascular space, yet melt or dissolve at the appropriate time. Research led to a type of medicinal suppository base (massa esterinum) composed of mixtures of mono-, di-, and tri-glycerides (Witepsol H15, H175, H37; Hüls America, Piscataway, NJ). These bases are insoluble in water and melt in the range of 33.5 to 36.5°C. Their constituent glycerides are normal elements in the blood stream; therefore, the stent is truly biocompatible.

To prepare the stent, the base material was melted in a water bath at 45°C and then drawn into glass micropipettes (Microcaps; Drummond Scientific, Broomall, PA) of varied internal diameters (0.195, 0.280, 0.340 mm) using capillary action. The glyceride casts were then allowed to cool at room temperature overnight. Prior to use, the stents were expelled from the micropipettes using appropriately sized wire plungers (Drummond Scientific).

The glyceride stents were used in conjunction with a sutureless, fibrin glue-based anastomosis. The glue was composed of two solutions [7], which were mixed on the artery at the time of anastomosis. The fibrin solution consisted of 0.25 g of bovine fibrinogen (ICN Biomedicals, Cleveland,



Fig 1. Various size glyceride stents. A = 1.2 mm; B = 0.6 mm; C = 0.28 mm; D = 0.34 mm; E = 0.195 mm. One grid cell = 1 mm<sup>2</sup>. ( $\times 9$ , before 14% reduction.)

OH) mixed with 2 ml of sterile water. The thrombin solution consisted of 1,500 U bovine thrombin (Sigma Chemicals, US), 0.25 ml of 0.5 mol/L calcium chloride (Sigma), 1,500 KIE of bovine lung-derived aprotinin (ICN Biomed), and 1.5 ml of sterile water. Both solutions were mixed in a 38°C water bath for at least 30 minutes prior to use.

*In vivo* testing of the glyceride stent involved 25 male Sprague-Dawley rats weighing between 380 and 480 g. After general intramuscular anesthesia using a mixture of ketamine (Ketaset; Aveco, Ft Dodge, IA) (112.5 mg/ml) and Promace (acepromazine; Aveco) (2.5 mg/ml) given intramuscularly at a dose of 0.07 ml/100 g body weight, the rats' groins were shaved and prepped. The choice of operative side was randomized.

Through a unilateral groin incision, the inferior epigastric artery was exposed, and its diameter was measured. It was then dissected free and relieved of spasm with 1% topical lidocaine. Any branches were tied off with 11.0 nylon suture (Ethicon, Somerville, NJ). The artery was placed in a double clamp, transected with straight microscissors, and irrigated with heparinized saline (10 U/ml). An appropriately sized stent (Fig 1) was then placed into one end of the cut vessel (Fig 2). Care was taken to select a stent small enough to easily slide into the vessel but large enough to provide good vessel edge apposition.



Fig 2. An 0.35 mm stent (arrow) placed in one end of cut vessel. Grid cell = 1 mm<sup>2</sup>. ( $\times 18$ , before 32% reduction.)



Fig 3. Both ends of vessel over stent, with central portion of stent visible. Grid cell = 1 mm<sup>2</sup>. ( $\times 12$ , before 50% reduction.)

The second vessel end was then placed over the stent (Fig 3), and the clamp arms were approximated, thereby closing the vessel over the stent (Fig 4). The fibrin glue was then applied using two 1 ml tuberculin syringes with 27-gauge needles (Monoject; Sherwood Medical, St Louis, MO). One drop of thrombin solution from one syringe was simultaneously applied to the vessel with one drop of fibrin solution from the other syringe. The vessel was left undisturbed for 5 minutes to allow the fibrin glue to set, after which 10 ml 45°C water was applied to the operative

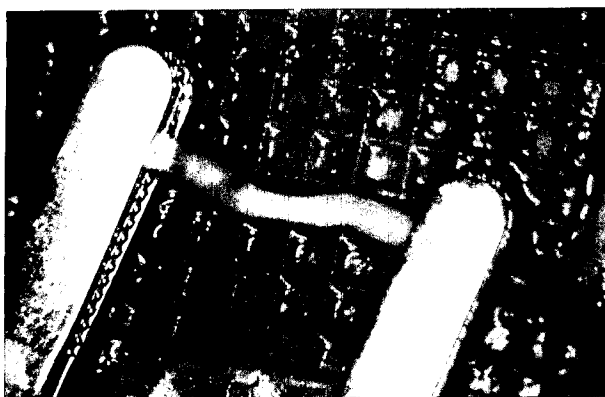


Fig 4. Vessel ends in excellent approximation, and it is ready for fibrin glue application. Grid cell = 1 mm<sup>2</sup>. ( $\times 9$ , before 50% reduction.)

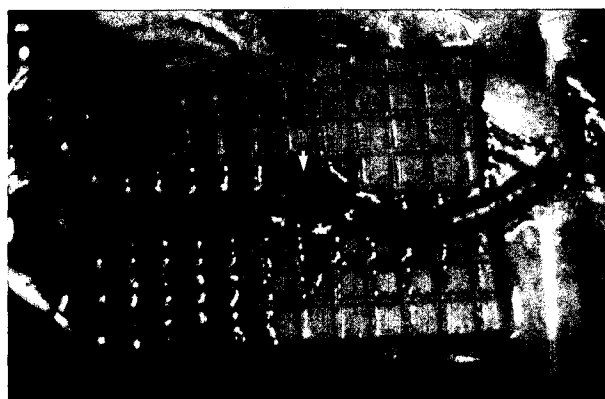


Fig 5. Completed anastomosis with good flow. F = fibrin glue. Grid cell = 1 mm<sup>2</sup>. ( $\times 6$ , before 50% reduction.)

site, thereby melting the stent. The proximal clamp was released for a moment to allow a small amount of blood into the anastomotic site.

The blood/glyceride combination was mixed with a gentle squeezing motion on the artery with the blunt end of a forceps. The distal clamp was then released, followed by the proximal clamp, thereby washing the melted, emulsified glyceride out of the vessel (Fig 5). The skin was closed, and the anastomosis was re-examined on postoperative days 1, 7, 14, and 30. Assessment consisted of the standard arterial milking test, as well as high-frequency pulsed Doppler ultrasound examination (VF-1; Crystal Biotech, Hopkinton, MA).

A control group of 14 rats was used. These rats were operated on and assessed in a similar fashion to the stented group except that instead of a fibrin-glue/glyceride stent anastomosis, a standard, 11-0 nylon (Ethicon), 4- to 5-stitch suture anastomosis was performed. All operations were

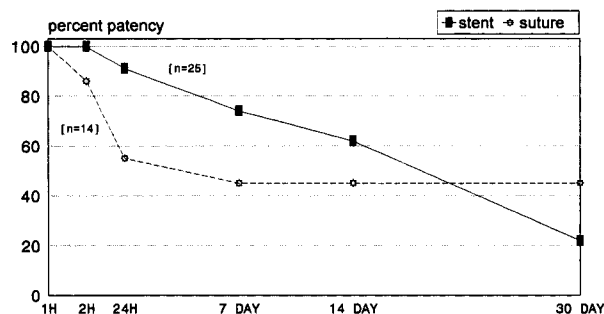


Fig 6. Patency rates: stent versus suture.

timed from either the point of first suture placement (control group) or the first grasping of the stent (study group), to the point of documented blood flow resumption with complete hemostasis.

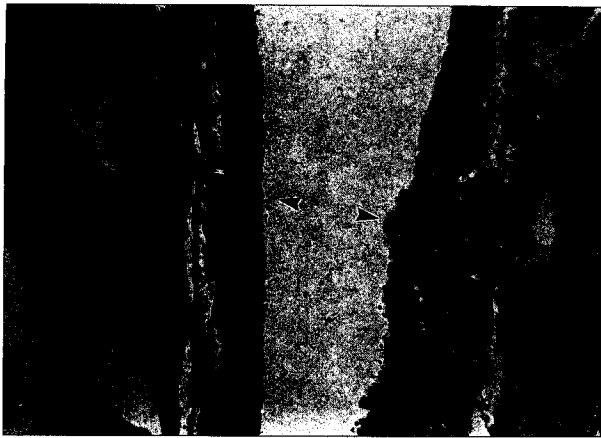
Histological specimens were taken at each examination interval. In addition, 1 specimen was taken at postoperative day 3. Specimens were removed from the rodent and immediately fixed in 10% buffered formalin for at least 72 hours. Specimens were then paraffin-embedded and sectioned. Staining consisted of standard hematoxylin/eosin stain, as well as Carstairs' stain for platelets and fibrin.

Institution guidelines for the care and use of animals were adhered to throughout these studies. Comparative statistical analyses consisted of student's t-tests (Microsoft Excel Version 4.0), with errors reported as standard deviation.

## Results

As shown in Figure 6, the 1- and 2-hour patency rates using the glyceride stent method were 100% (25 of 25). At 24 hours, the rate was 91% (20 of 22). At 1 week, histological specimens demonstrated profound aneurysm formation, and patency rates dropped to 74% (14 of 19). At 14 days, the aneurysms had grown larger, and patency rates decreased to 62% (10 of 16), whereas at 30 days, 23% (3 of 13) of the anastomoses were patent.

Histological examination of the glyceride-stented arteries showed the excellent vessel edge apposition afforded by this technique (Figs 7A, B; 8). A 3-day specimen (Fig 9) demonstrates aneurysm formation with continued vessel patency.



A



B

Fig 7. (A, B) Stented anastomosis 1 hour postoperatively. Arrowheads mark repaired vessel edges. F = fibrin glue. (H & E stain  $\times 200$  [A],  $\times 450$  [B], before 50% reduction.)



Fig 8. Repaired vessel after 24 hours. Anastomotic line at arrow. (H & E  $\times 450$ , before 50% reduction.)



Fig 9. Stent-repaired vessel after 3 days. Note the patent but developing aneurysm. (H & E  $\times 100$ , before 50% reduction.)

Figure 10A, B shows a patent 30-day specimen with moderate aneurysmal dilation. A breakdown of the normal medial wall architecture is seen, with disruption of the elastic lamellae and hyperplasia of the subintima. Figure 11A, B displays another 30-day specimen, with disruption of the medial architecture and the elastic lamellae evident in both low- and high-power views.

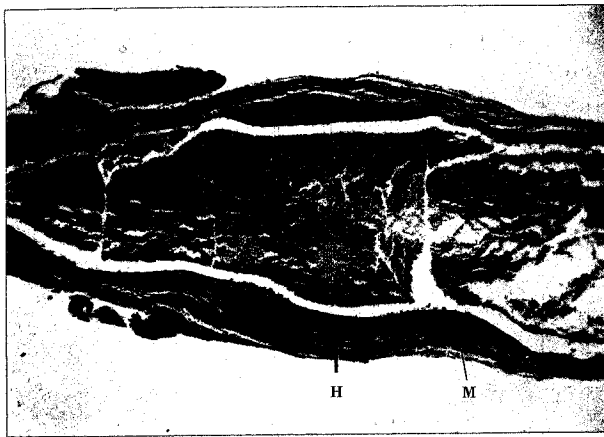
The patency results of the control suture group are seen in Figure 6. One hour postoperatively, all vessels were patent, whereas at 2 hours, 2 of the 14 vessels had closed (86% patency). At 24 hours, 55% patency was observed, which dropped to 45% at 7 days. Patency continued at 45% through days 14 and 30.

The average time to complete a stented/glued anastomosis was 18 minutes ( $\pm 4$  SD), whereas

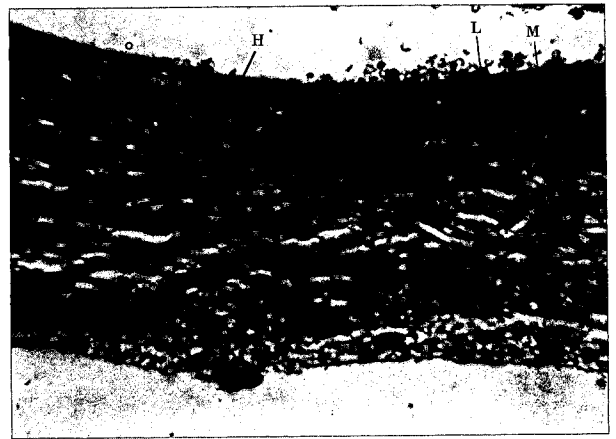
the sutured anastomosis required 28 minutes ( $\pm 6$  SD), which is a statistically significant difference ( $p < 0.0001$ ).

### Discussion

Although the use of stents in vascular surgery dates to the 19th century, we document the first time they have been used on this small scale. Although fine sutures have provided the basis of microsurgical technique since its inception, suture technique is limited by the size of the vessel under study. The smaller the vessel, the greater the percentage stenosis and deformity caused by the sutures. Although the change in luminal diameter of a repaired human aorta may be im-

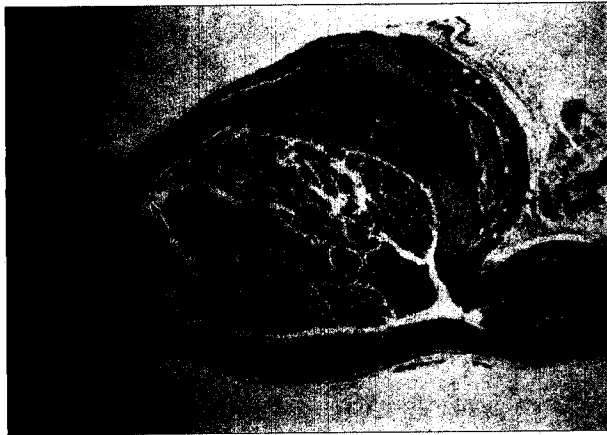


A



B

Fig 10. (A, B) Repaired vessel 30 days postoperatively. Note the moderate aneurysmal dilation. Normal media (M) and lamellae (L) give way to subintimal hyperplasia (H). (Carstairs' stain  $\times 65$  [A],  $\times 200$  [B], before 50% reduction.)



A



B

Fig 11. (A, B) Large, patent aneurysm after 30 days, with disruption of normal media architecture (D). Note the subintimal hyperplasia (H). (Carstairs' stain  $\times 40$  [A],  $\times 100$  [B], before 50% reduction.)

perceptible, each suture in the repair of a 0.5-mm vessel slightly reduces the vessel's diameter, no matter how well the anastomosis is performed.

Use of a temporary stent and a sutureless technique may obviate this problem. Because no sutures are placed, the limiting factor becomes the size of the stent itself. We have shown that stents of less than 0.2 mm are easily produced. But the stent technique clearly has its difficulties, as reflected in the 30-day patency rate of 23%.

Aneurysm formation is the most likely cause of the low 30-day patency rate. This aneurysm formation, however, is not unique to this experimental method. Maxwell and colleagues [9] described a 60% aneurysm rate in standard, sutured rat femoral anastomoses. These aneurysms were

smaller than those in our experiment and did not affect the patency rate. However, theirs and others' [10] histological findings of medial necrosis, subintimal hyperplasia, and, most importantly, loss of elastic lamellar structure are identical to those presented herein.

The most likely reason for the high aneurysm-related failure rate in this study is the poor healing of the media and the elastic lamellae, which is brought on by the constant pressure of the arterial system on the fibrin glue. Despite adequate strength to prevent bursting, fibrin glue lacks the durability to hold the media in close enough apposition to allow proper healing over time. Sutures, in contrast, lose a minimal amount of their strength over time and keep the media

better approximated during the postoperative healing period. The fibrin glue-based anastomosis ultimately fails late (7–30 days) because local flow aberrations in the aneurysmal area thrombose the vessel. Sutured anastomoses, in contrast, fail *early* because the technical difficulty of suture placement causes intimal damage and stenosis and results in early (2–24 hr) thrombosis of the vessel.

Our laboratory is currently exploring ways to circumvent the fibrin glue weakness. One method under study is to increase the fibrin glue's strength by the simple addition of coagulation factor XIII. Factor XIII, or fibrin cross-linking factor, is found in normal plasma, but it is absent from our commercially derived preparation. (The factor was not commercially available at the time of this experiment, although it is made by Immuno Research, NY). This omission has been cited [11] as a potentially serious flaw in fibrin glue preparation, which could yield suboptimal fibrin mesh strength. The inclusion of factor XIII may yield a better long-term patency. Likewise, transforming growth factor- $\beta$  has been shown to increase postoperative intra-abdominal adhesion formation in rats. If the current theory on adhesion formation is correct and these adhesions form from incompletely degraded fibrin aggregates, it should be possible to intentionally inhibit fibrin degradation and promote a more permanent fibrin clot.

Another method to decrease aneurysm formation could be the use of a cuffed end-to-end anastomosis. By cuffing the anastomosis, a ring of vessel wall would surround the anastomosis and afford an extra degree of protection from the constant outward forces of the arterial system.

The stented/fibrin glue-based system is significantly faster than standard suture techniques. Although a sutured anastomosis required an average of 28 minutes, a stented/glued anastomosis took only 18 minutes; 5 of those minutes spent waiting for the fibrin to cure. Therefore, during this cure time, another vessel could be started, and the anastomosis of two 0.35-mm vessels could be performed in less than 25 minutes.

The glyceride stent is only one of several new techniques our laboratory is experimenting with. We are currently working on a two-staged stent for use with laser-assisted anastomoses. The cen-

ter of the stent is composed of polyethylene glycol (PEG) 4000, a water-soluble, biocompatible material with a melting point of approximately 65°C. The PEG core is covered with a water-insoluble, glyceride coating. The PEG stent is placed in an artery analogous to that described for the glyceride stent; however, laser energy is used in place of fibrin glue to secure the anastomosis. The laser energy melts the outer glyceride coating but leaves the inner PEG intact to support the lumen during the laser process. After laser completion, the clamps are released and the blood flow dissolves the water-soluble PEG center. Preliminary *in vivo* tests are ongoing.

Use of dissolvable stents in modern microsurgery is still a technique in its infancy. We believe that with further research, these techniques and others will someday have an active part in the practice of microsurgery.

---

The authors thank Ms Lynne Bonavita for her help, and The Plastic Surgery Educational Foundation and Dr J. N. Cunningham for their support.

---



---

## Open Discussion

---

*Dr William Swartz (Pittsburgh):* I think it is fascinating technology, but the model seems a little bit at variance with some of the published reports of suture models. Typically, a high-quality suture model is patent at 90% plus at a week, not 45% as your controls would show. Do you have an explanation for that?

*Dr Moskovitz:* The average suture anastomosis used is the rat femoral artery, which is roughly twice the diameter of what we are talking about here. The other thing is that obviously I was performing both of these and I do not have as much experience as most of our microvascular surgeons here. But one of our microsurgeons did perform several anastomoses on these, and preliminary results show roughly a 70 to 80% patency at 1 day, which dropped off a little bit, maybe to 60 to 70% at 1 week and then remained steady through that period. So, there is room for improvement. We think that since the flow with the fibrin glue anastomosis weakens over time, if

we can get over that problem, we probably would have a better technique for these smaller vessels.

Dr Swartz: One second comment I have about anastomotic aneurysms is that it seems to be a problem in rat femoral arteries, but to my knowledge is not a problem in human microvascular surgery, and I think it is model-dependent.

Dr Moskovitz: Yes. Several papers by Buncke and Maxwell have noted that also and that will be a plus for this.

## References

- 1 Abbe R. The surgery of the hand. *NY Med J* 1894;14:33-39
- 2 Carrel A. La technique operatoire des anastomoses vasculaires et la transplantation des visceres. *Lyon Med* 1902; 98:859
- 3 Obora Y, Tamaki N, Matsumoto S. Nonsuture microvascular anastomosis using magnet rings. *Surg Neurol* 1978; 9:117-119
- 4 Daniel RK, Olding M. An absorbable anastomotic device for microvascular surgery: experimental studies. *Plast Reconstr Surg* 1984;74:329-336
- 5 Coleman DJ, Timmons MJ. Non-suture external cuff techniques for microvascular anastomosis. *Br J Plast Surg* 1989;42:550-555
- 6 Östrup LT, Berggren A. The Unilink instrument system for fast and safe microvascular anastomosis. *Ann Plast Surg* 1986;17:521-525
- 7 Kamiji T, Maeda M, Matsumoto K, et al. Microvascular anastomosis using polyethylene glycol 4000 and fibrin glue. *Br J Plast Surg* 1989;42:54-58
- 8 Cong Z, et al. Experimental study on microvascular anastomosis using a dissolvable stent support in the lumen. *Microsurgery* 1991;12:67-71
- 9 Maxwell GP, Szabo Z, Buncke HJ Jr. Aneurysms after microvascular anastomoses. Incidence and pathogenesis in experimental animals. *Plast Reconstr Surg* 1979;63:824-829
- 10 Baxter TJ, O'Brien BMcC, Henderson PN, Bennett RC. The histology of small vessels following microvascular repair. *Br J Surg* 1972;59:617-623
- 11 Saltz R, Sierra D, Feldman D, et al. Experimental and clinical applications of fibrin glue. *Plast Reconstr Surg* 1991;88:1005-1017