Eur J Vasc Endovasc Surg **28**, 526–533 (2004)
doi:10.1016/j.ejvs.2004.07.018, available online at http://www.sciencedirect.com on SCIENCE DIRECT®

Sutureless Anastomoses of Small and Medium Sized Vessels by Medical Adhesive

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Purpose. *Using an animal model we have assessed sutureless anastomoses.*
Methods. *The two cut ends of the rabbit common carotid artery were sutured by three stitches with a 120° interval circumferentially, then two optional threads were pulled horizontally and 0.1 ml adhesive was smeared on the attached surface of the two ends. The three stitches were removed after completion of anastomosis. The burst pressure of the anastomosis was measured and compared with that of a traditional sutured artery.*
Results. *The glued anastomosis was associated with: a shorter completion time (8.25±6.34 min vs. 20.67±14.24 min, P< 0.01), less bleeding (3.17±9.04 ml vs. 11.04±16.28 ml, P<0.01), and equivalent patency (93.8 vs. 87.5%, P>0.05). The sutureless anastomosis was associated with less intimal thickening (decreased by 31.4, 24.5, 23.9 and 31.9%, P<0.01 compared with the traditional suture group at 1, 2, 4 and 12 weeks, respectively).*
Conclusion. *Glued anastomoses provides an effective, simple and feasible way for anastomosing small or medium caliber vessels. This technique may reduce intimal injury.*

Keywords: Intimal hyperplasia, vascular; Anastomtic stenosis, vascular; Vascular anastomosis; Sutureless; Medical adhesive.

Introduction

Anastomosis of small vessels is now widely carried out in vascular surgery. However, anastomotic stricture remains one of the major causes for graft failure. Traditional suturing anastomosis can be associated with intimal injury vascular wall ischaemia or foreign body reaction. Therefore, surgeons have been searching for a better anastomotic method with easier manipulation, minimal complication and higher patency. Early in 1889, Jassinowski[1] pointed out that 'vascular sutures should not penetrate and damage the intima of the vessel'. In order to minimize injury to the vascular intima, most researches focused on the study of Vascular Closure Staple (VCS),[2–4] a non-penetrable anastomosis technique. But VCS has not been accepted by surgeons because of high cost, complexity, special skills, long mobilization of the vascular segment between the anastomotic ends and necessity of similar diameters of the two anastomotic vascular ends. The introduction of medical adhesive provided another anastomotic technique. Most researchers use bio-glue as adhesive and intravascular stent or balloons as support, and some others use medical adhesive as enhancement after sleeve anastomosis.[5–7] Bio-glue is mainly used for haemostasis. Chemical adhesive is seldom used in vascular anastomosis. The procedure of endovascular stent support is tedious and complicated. The sleeve anastomosis is not often applied because of its strict indication and poor patency. Therefore, using chemical adhesive, we designed the sutureless anastomosis on the basis of 'three-spot anastomosis' without endovascular stent or sleeve-encasement, and compared it with the conventional suturing anastomosis.

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Methods

Animals

Eighteen male New Zealand white rabbits weighing 2.5–2.8 kg with a qualified certificate were purchased from the Experimental Animal Center of Zhongshan Hospital, Fudan University. Each animal was raised

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with food and water in a standard cage individually. Two of them were used for the feasibility study of sutureless medical adhesive anastomosis. The remaining 16 animals were equally divided into study group and control group. Bilateral *in situ* dissection and anastomoses of common carotid arteries were performed in each animal, using sutureless medical adhesive anastomosis for study group and conventional suturing anastomosis for control group.

In situ end-to-end anastomosis of the common carotid artery in rabbits

The animal was anesthetized with Ketamine (60–100 mg/kg) and Diazepam (6–10 mg/kg) intramuscularly and then fixed on the operating table with the neck over-stretched. A 6 cm long incision in the neck was made, the platysma and anterior cervical muscles were transected and the right common carotid artery was mobilized for approximately 5 cm. Then the carotid artery was transversely cut between interruption clips. In the control group, interrupted end-to-end anastomosis was performed using a 7-0 non-absorbable suture. In the study group, the anastomosis was carried out using medical adhesive (0.5 ml/ampule, product of Beijing Suncon Medical Adhesive Co. Ltd). The medical adhesive used in this experiment is made of cyanoacrylate, catalyst and auxiliary components. It is transparent and flowing. When coming into contact with blood it polymerizes into a solid substance with the shape of a glue membrane. The adhesive intensity for human tissues is 3.45 kg/cm. The two ends of the artery were sutured with three stitches at 120° intervals circumferentially, then two of the three sutures were gently pulled horizontally and 0.1 ml medical adhesive was smeared on the attached outer surface of the two ends. The two sutures were maintained in tension for 10–15 s, thus one third of the anastomosis was finished. The other two thirds were treated in the same manner, but before final adhesive smear, heparin saline was flushed into the anastomosis to ensure no adhesive was present in the lumen. When the other two thirds were completed, the distal mini-clip was removed first and the anastomosis was checked by the retrograde blood flow across the anastomosis, then the proximal clip was removed. The three sutures were drawn out to complete the sutureless anastomosis. The distal pulsation was routinely examined to verify the anastomotic patency. After finishing the right side, the left side was finished in the same way. The incision was closed layer by layer after thorough hemostasis. The operation time and bleeding amount of each anastomosis was documented. Each animal was given 3 mg/1 mg/kg standard heparin subcutaneously postoperatively. They were fed in isolated cages in a clean and well-ventilated room with comfortable temperature (20–24 °C).

Anastomotic break pressure measurement of adhesive anastomosis

The adhesive anastomosed common carotid artery was cut off and rinsed with heparin saline. Then, with the vascular anastomotic break pressure-measuring instrument (see illustration), the anastomotic break pressure was measured. One end of the artery was connected to a plain needle and fixed with thread, the other end was clipped with a hemostat. The needle was linked to a blood pressure apparatus and a pressure syringe through a triplet and blood transfusion catheter. The anastomosed artery was irrigated continuously with saline. When the saline in the syringe was simultaneously injected into the artery and the catheter linking to the pressure apparatus, the pressure number read from the apparatus reflected the pressure-bearing of the anastomosis. The anastomotic break pressure was documented.

Histologic study and morphometry

1, 2, 4 and 12 weeks postoperatively, the animals were anesthetized as before and both common carotid arteries were excised. The specimens were immersed in 10% neutral formaldehyde for light microscopy and immunohistochemical analysis, and in 2.5% glutaraldehyde for electromicroscopy. The formaldehyde

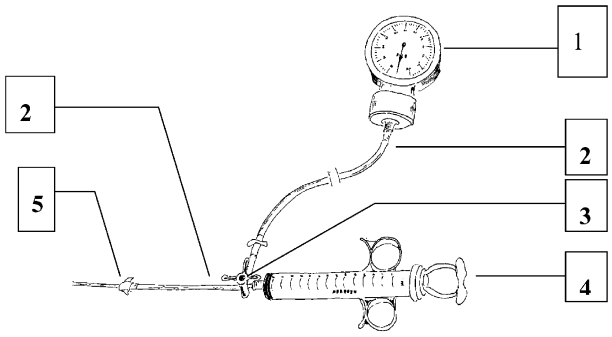

Illustration. Vascular anastomotic break pressure measuring instrument. 1. blood pressure apparatus (product of Shanghai Medical Instrument Factory); 2. connecting tube (80 cm in length, 2.0 mm in diameter, part of blood transfusion tube); 3. three-way cock (a product of Jingling Medical Instrument Co., Ltd, Hangzhou); 4. pressure syringe (20 ml, a product of medtronic Company); 5. measuring tube (18 G needle with plain tip, a product of Shanghai Medical Instrument Factory).

fixed specimens were hydrated, paraffin embedded, sliced into 4 μm sections, then stained with hematoxylin-eosin and elastin van Gieson. The stained sections were observed under light microscope, and with the aid of imaging analyzing system (Product of Automatic Technology Company of the Chinese Academy of Sciences), the intimal thickness, medial thickness and their comparative ratios, the intimal area, medial area and their ratios were measured and calculated.

Electron microscopy

The fixed specimens were rinsed with buffer and dehydrated gradually with alcohol, then treated with acetone and dried with carbon dioxide. After spray of target gold, they were observed under scanning electron microscope (S-520, product of Hitachi Company). For transmission electron microscopy, the fixed specimens were rinsed with buffer, fixed with osmate, dehydrated gradually with alcohol, embedded with Poly/Bed 618, sliced longitudinally and stained with plumbum, then observed under transmission electron microscope (Philips-CM*120, product of the Netherlands).

Immunohistochemical analysis

The paraffin specimens of the study and control groups harvested at 2 weeks postoperatively were sliced into 4 μm sections. The proliferating cells were labeled with proliferating cell nuclear antigen (PCNA) antibody by Streptavidin-peroxidase (SP) method, which was conducted according to the instruction of the Test Kit. The expression of PCNA was observed under light microscope. With the imaging analysis system, the total number of cells and the number of PCNA positive cells in tunica intima and tunica media were documented. The proliferating cell ratio (PCNA positive cells/total cells) in tunica intima or tunica media was named as cell proliferating index (PI). Similar to that of PCNA, transforming growth factor-β1 was also immunohistochemically analyzed.

Statistical analysis

Data are expressed as mean ± standard deviations. Anastomosing time and blood loss between the groups were compared by use of Students' *t* test. Patency rates and proliferating indices between the groups were compared by use of the chi-square test. Neointima thickness between the groups was compared by use of the Wilcoxon rank sum test. Differences were considered significant at $P<0.05$.

Results

The technical procedure of end-to-end sutureless anastomosis with medical adhesive in rabbit common carotid artery was feasible. The average anastomotic break pressure of the rabbit common carotid artery was 58.7 kPa. The mean anastomosing time of each anastomosis was 8.19 ± 6.51 min in the study group and 20.50 ± 14.35 min in the control group ($P<0.01$). The mean blood loss in each anastomosis was 2.44 ± 5.83 ml in the study group and 10.38 ± 17.49 ml in the control group ($P<0.01$). All animals survived without occurrence of hemiplegia or neck twist. During specimen harvesting, one anastomosis was found to be occluded by the medical adhesive inside the lumen at 1 week. All the other anastomoses were patent with a patency rate of 93.8% (15/16). In the control group, one anastomosis at week 4 and one at week 12 were found to be occluded with thrombi inside, and the remaining anastomoses were patent with a patency rate of 87.5% (14/16) ($P>0.05$). No hematoma or rupture or local dilation or aneurysm formation was detected in both groups.

Histologic study showed that the neointima became thicker with time lapsing, reaching the peak at 2–4 weeks and then remaining relatively stable. No further thickening of the neointima was observed at 12 weeks, though it became denser because of more deposition of extracellular matrix and less proliferation of smooth muscle cells (SMCs). The degrees of intimal hyperplasia were quite different, lighter in the study group (see arrows in Fig. 1). Compared with the control group, the intimal thickness in the study group decreased by 31.4%, $P<0.01$, 24.5%, $P<0.01$, 23.9%, $P<0.01$, and 31.9%, $P<0.01$ at 1, 2, 4 and 12 weeks, respectively (see Table 1). So was the intima/media ratio, the intimal area and the intimal/medial area ratio. No significant difference was found in medial thickness comparison or medial area comparison.

Under scanning electron microscope, the sutures in the vascular wall of the control group looked like dense grass (see arrows in Fig. 2). And the vascular wall of the anastomosis looked markedly thickened (see arrows in Fig. 3). The thread was surrounded by endothelial cells in whirlpool shape from remote areas. Though the thread was covered thoroughly by endothelia, the needle holes of the suture usually looked like traps, forming great ups and downs between the thread projections and the thickened vascular wall (see arrows in Fig. 4). In the study group,

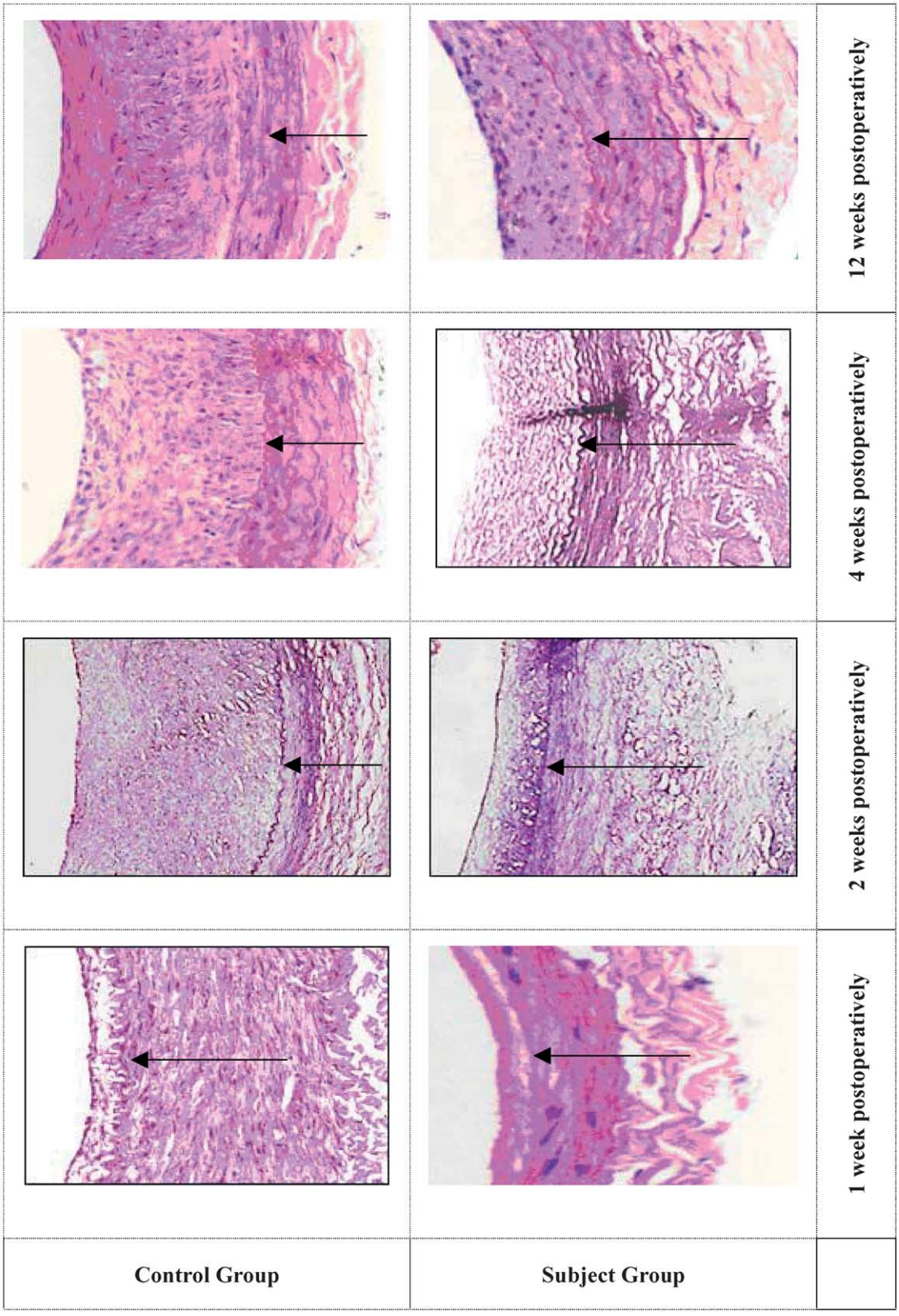

Fig. 1. Light Microscopy: the arrow indicates the intimal hyperplasia (HE staining ×40).

Table 1. Comparison of intimal thickness between Control and Subject Groups ($\bar{X}\pm S$, μm)

Group	1 week postop.	2 weeks postop.	4 weeks postop.	12 weeks postop.
Control	35±7	53±9	67±12	69±15
Subject	24±4	40±3	51±7	47±8

$P<0.01$.

no thread was seen and the luminal surface was comparatively smooth. The anastomotic region was mostly endothelialized at 4 weeks, the anastomosis was endothelialized from both sides and the surface was comparatively smooth (see Fig. 5). The endothelialization was completely at 12 weeks, when the luminal surface was intact and smooth; no projection lesion or cystic dilation was seen.

Under transmission electron microscope, most SMCs in the neointima of the control group were synthetic phenotype. Abundant rough endoplasmic reticulum lied in the cytoplasm. Chromatin aggregated in the nuclei and became larger (see Fig. 6). In the study group, most SMCs arranged in a relatively messy state and presented as contractile phenotype, some of which were hypertrophic, with little rough endoplasmic reticulum and much fibrin component (see Fig. 7).

Immunohistochemical analysis showed more PCNA positive SMCs in the intima of the control group with its PI of 36.35±0.72, and a decreased PCNA positive SMCs in the media with the PI of 12.23±2.17 ($P<0.01$) (see Fig. 8). Less PCNA positive SMCs were detected in the subject group, the PI of intima and media was 9.12±2.26 and 3.09±0.08, respectively, $P<0.01$ (see Fig. 9). Variant expression of TGF-β1 was detected in both groups at 2 weeks. TGF-β1 positive SMCs were found in both intimal and medial areas in the control group (see Fig. 10), while some weak positive SMCs were restricted only in the intimal area in the study group (see Fig. 11).

Discussion

Early in 1955, Samuels[8] attempted to perform anastomosis of small vessels with metal clips. After that, many new methods were reported, for example, connecting the cut ends with absorbable apparatus,[9] fusing the small vessel ends with laser apparatus,[10,11] anastomosing the vessels with vascular stapler.[12,13] Although they had some advantages over the traditional methods, their disadvantages were also apparent: the operating system and manipulation process were complicated, the indication was limited, the cost was high and the hard foreign bodies had to be left in the vascular anastomosis. Therefore, the above vascular anastomosis methods were not widely accepted and applied clinically. Exploration of new

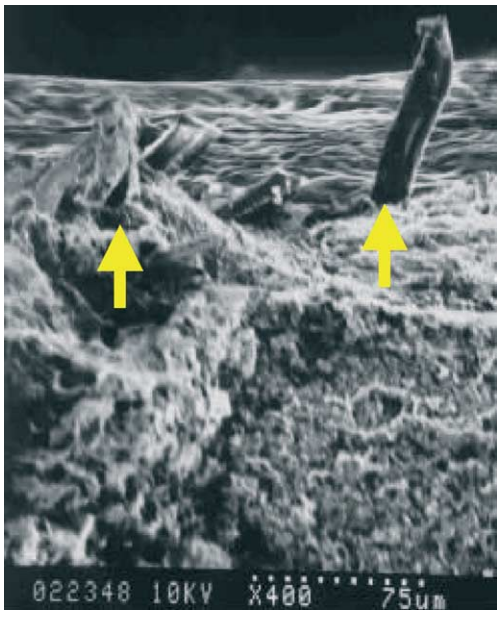

Fig. 2. Control group: threads distributed like grass in the vascular wall (×400).

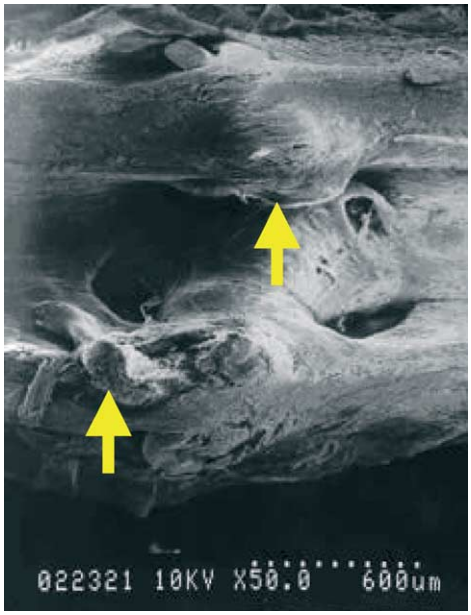

Fig. 3. Control group: the anastomosis is narrow with threads inside, and the anastomotic vascular wall is thick (×50).

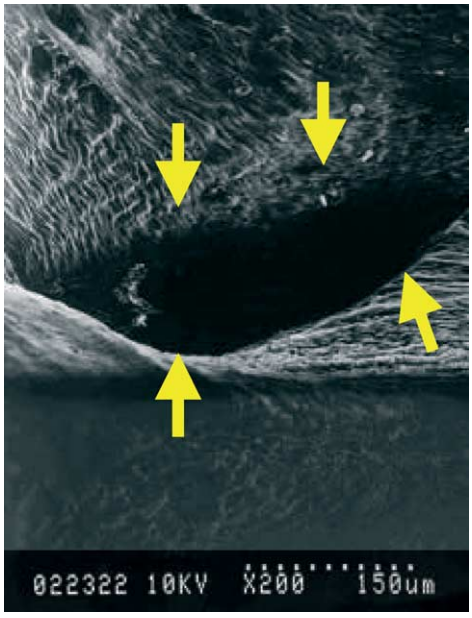

Fig. 4. Even the thread has been covered thoroughly by endothelia, the needle-hole of the suture usually remained trap-like, which resulted in great ups and downs between projection thread and thickening vascular wall (×200).

anastomotic methods became a focus of attention. Gottlob *et al.*[14] conducted sleeve anastomosis in small arteries and veins with enhancement of the anastomosis by bio-fibrin glue in 1968. This method minimized vascular wall injury by reduction of needle suture, but the operation procedure was tedious and

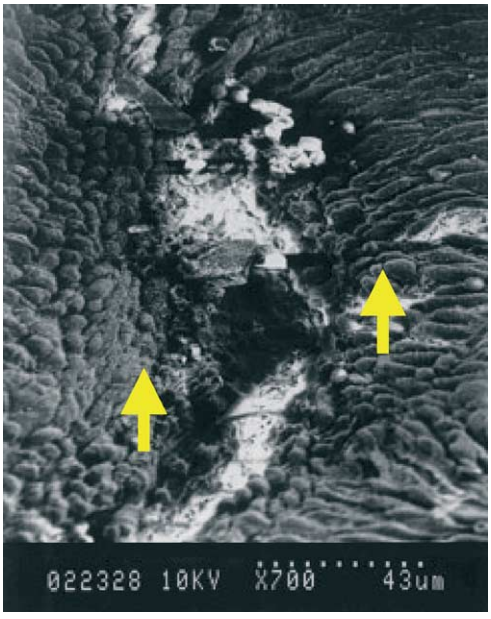

Fig. 5. Subject group: the anastomotic region was most endothelialized at 4 weeks, the anastomosis was approximation-covered by endothelial cells from both sides and the surface was comparatively smooth (×700).

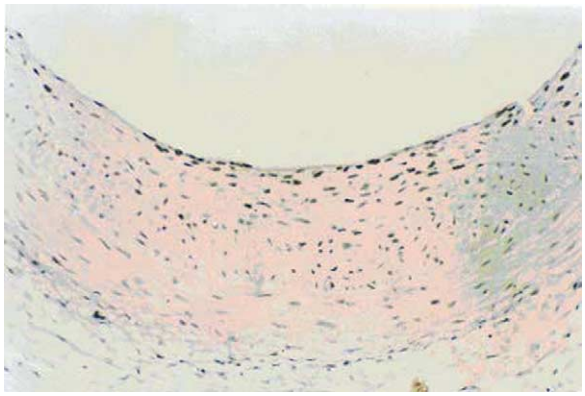

Fig. 6. Control group: SMCs proliferated actively and the shape of dumb-bell implicated the process of mitosis, abundant tubular and vesicular rough endoplasmic reticulum lied in the cytoplasm (×15,000).

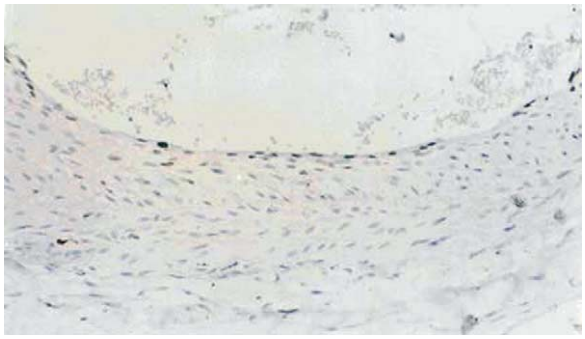

Fig. 7. Subject group: most SMCs arranged in a relatively messy state and presented as contractile phenotype (×2000).

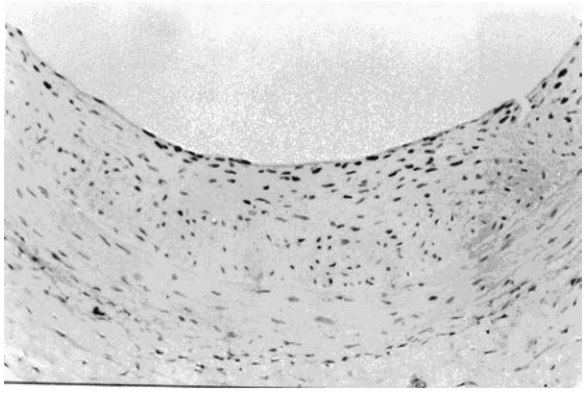

Fig. 8. Immunohistochemical analysis of PCNA: abundant PCNA positive cells in the neointima at 2 weeks postoperatively in the Control group (×200).

time costing. Especially when the vessel was a small calibre vein or the two ends of the vessels were almost the same in diameter, the procedure would be more difficult. In this study, we designed a sutureless medical adhesive anastomosis method, which combines the advantages of the traditional anastomotic technique and the bio-fibrin glue anastomotic technique. The

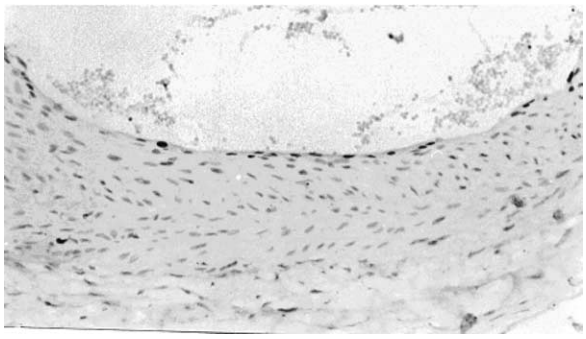

Fig. 9. Immunohistochemical analysis of PCNA: less PCNA positive cells in the neointima at 2 weeks postoperatively in the Subject group (×200).

minimal needle suturing or no needle suturing technique can surely eliminate vascular injury and foreign body injury in the vessel. As the two vascular ends are anastomosed by medical adhesive, anastomotic bleeding can safely be prevented. This technique can be used in small and medium-sized vessels, and in vessels whose diameters are different and small veins with thin walls and narrow lumens.

In order to minimize foreign effect at the anastomotic site and to eradicate possible factors affecting anastomotic intimal hyperplasia, three traction threads were drawn out after completion of anastomosis. Then the question arises: whether the anastomosis without suture support is liable to rupture with blood flow pressure. Actually, the answer mainly depends on the endovascular blood pressure and the sustaining pressure of the anastomosis. Human physiologic blood pressure is 12.0–16.0 kPa, and may rise to 39.0 kPa or more in some rare pathological conditions. The mean burst pressure of the anastomosis obtained in this experiment is 58.7 kPa, which is much higher than that of the pathologic blood pressure. This implies that the sutureless adhesive anastomosis of vessels is safe and feasible.

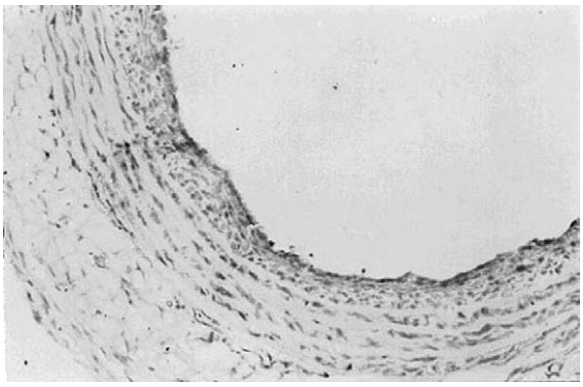

Fig. 10. Immunohistochemical analysis of TGF-β1: abundant TGF-β1 positive cells in the neointima and medial tunica at 2 weeks postoperatively in the Control group (×200).

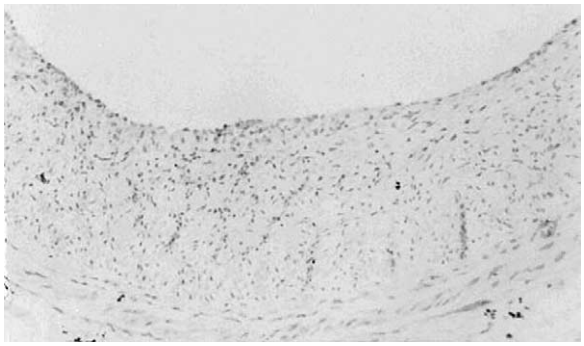

Fig. 11. Immunohistochemical analysis of TGF-β1: less TGF-β1 positive cells in the neointima near the lumen at 2 weeks postoperatively in the Subject group (×200).

The comparative study suggests that the sutureless anastomosis has more advantages over the traditional method. The anastomosing time was shortened and the bleeding amount was decreased. The patency rates were higher (though not significant statistically). Unfortunately, the over-dosed adhesive around the anastomosis leaked into the inside of the lumen and led to anastomotic occlusion in one case of the study group. Accordingly, the adhesive applied on the anastomotic site should not be excessive. All the examinations including the gross specimen observation and micro-morphological studies indicate a lighter injury and a higher patency of the anastomosis in the sutureless adhesive group compared with those of the control group. The possible reasons lie in: avoidance of the transmural injury by the needle and suture; elimination of the proliferative reaction by the foreign bodies such as suture; and provision of a less interference with the blood dynamics by the smooth lumen of the anastomosis.

The immunohistochemical results showed that the PCNA expression and PI calculation in the study group were significantly lower than those in the control group, suggesting that the sutureless adhesive anastomosis method brought lighter injury to the vascular wall and thus induced less degree of VSMC proliferation. TGF-β1 was also selected for analysis because its expression in the vascular wall could reflect the degree of endothelialization. It is known that in vitro TGF-β1 can inhibit the growth of endothelia by changing the binding ability of endothelial growth factor (EGF), competitively suppressing the EGF induced growth-modulating gene expression,[15] and reacting with acid and basic fibroblast growth factors.[16] Therefore, the higher expression of TGF-β1 in the vascular wall indicates poorer endothelialization in the lumen. While lower expression of TGF-β1 in the vascular wall indicates a high degree of endothelialization of the lumen,

meaning less injury of the endothelia or good recovery of the endothelia. The weak positive expression of TGF-β1 limited in the intimal tunica in the study group indicates that the sutureless adhesive anastomosis brought lighter injury to the vascular endothelia.

The above indicated that the conventional suturing resulted in great impairment to the vascular wall, and the unabsorbable thread as a foreign body not only stimulated proliferation of the vascular wall but also produced a rough surface of the canal; the undulate lumen was prone to form turbulent flow, which promoted anastomotic stenosis and occlusion.

In conclusion, we think the sutureless anastomosis method of small vessels is feasible. It not only avoided transmural injury at the anastomotic site by the needle and suture, but also eliminated proliferation reaction by foreign bodies. This work provides an effective and simple method for end-to-end anastomosis of small caliber arteries, but it still needs further study for end-to-side anastomosis and artery-vein anastomosis.

Acknowledgements

We are grateful to Beijng Suncon Medical Adhesive Co., Ltd, for provision of the medical adhesive. This research is supported by the Youth Found of Zhongshan Hospital, Fudan University.

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Accepted 22 July 2004

Available online 27 August 2004