

## Anastomosis of Vessels less than 2 mm with the Vascular Clip System Clip Applier

Sutures may cause endothelial trauma and occlusion. The vascular clip system (VCS) clip applier may minimize endothelial injury. Fourteen carotid arteries of nine adult rabbits were transected and re-anastomosed with either #7-0 polypropylene (Group I, n=8) or VCS clips (Group II, n=6). The animals were sacrificed at 1, 3, 8, 14, and 30 days postoperatively. The operation time and bleeding amount were checked for each anastomosis. Carotid angiograms, photography, H&E staining and scanning electron microscopy (SEM) were performed. Fibrin and thrombus, inflammatory cell infiltration, endothelial disruption, luminal distortion, fibrosis, and wall thickening were compared. The luminal diameter was greater in group II. There were minimal differences in thrombosis, wall thickening and fibrosis between the two groups. However, fibrin, inflammatory cell infiltration, multinucleated giant cell formation, endothelial disruption, and luminal distortion were greater in group I. On SEM, group I showed trans-mural penetration. In contrast, group II showed suture margin eversion and no transmural penetration. Stenosis was greater in group I than in group II on carotid angiogram. The operation time was shorter in group II than in group I, i.e.  $5 \pm 1.4$  min vs.  $11 \pm 3.8$  min, respectively. The current data showed similar or superior results with VCS clips in comparison to conventional suturing with polypropylene.

**Key Words :** Sutures; Carotid Arteries; VCS Clip Applier; Anastomosis, Surgical; Vascular Staples

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## INTRODUCTION

With growing need for coronary artery bypass procedures, demands for greater cost efficiency and better long term results have increased. As a result technically more straightforward and less injurious methods of coronary artery anastomosis with superior patency rates have been sought. This was pertinent not only to reduce bypass time but also to make easier newly evolving technologies such as minimally invasive direct coronary artery and off pump coronary artery procedures. The Vascular Clip System® (VCS) clip applier, is purportedly an atraumatic, non-transmurally penetrating and semi-automated vascular suturing device, with the potential to effectuate quick and less traumatic automated suturing of fine vessels (1). The aims of the current study were 1) to assess the feasibility of performing anastomosis of vessels less than 2 mm with the VCS clip applier, and 2) to compare the surgical results using the VCS clips with standard polypropylene sutures at various postoperative phases.

## MATERIALS AND METHODS

### Animal preparation

All animals used in this study received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication no. 85-23, revised 1985). Fourteen carotid arteries of 9 fully grown rabbits weighing 3.5 to 4 kg were used. After pretreatment with atropine sulfate, the animal was placed in a specially designed restraining device. Anesthesia was then induced with 0.5 mL of xylazine and 2 mL of ketamine by intramuscular injection. Anesthesia was maintained through slow continuous intravenous dripping of 500 mg of penthotal sodium (mixed in normal saline). In the first 4 animals, the carotid artery was used from only one side. After familiarization with the surgical technique, both carotid arteries were used. The vessels were exposed through a longitudinal midline neck incision. Once the carotid ar-

tery was isolated, the animal was heparinized with 1,000 units of beef heparin injected intravenously. Clamps were placed proximally and distally to the segment of the carotid artery to be operated. The artery was transected and reanastomosed with either running sutures of 7-0 prolene® (Ethicon Limited, Edinburgh, U.K.) (group I) or with multiple interrupted staples using the VCS clip applicator (USSC, Auto Suture International, Inc. Norwalk, CT, U.S.A.) (group II). Six carotid arteries were anastomosed with VCS clips and 8 with 7-0 prolene sutures. The animals were sacrificed at 1 (n=4), 3 (n=1), 8 (n=1), 14 (n=1), and 30 days (n=2) postoperatively.

### Surgical technique

The VCS clip applicator has spaced, arcuated arms mounted on self-releasing application forceps containing 25 staples each. The long neck of the applicator rotates 360 degrees, allowing steady positioning of the surgeon's hand throughout the procedure for application of the staple. The 0.9 mm (small) sized clips were used in the current study (Fig. 1). The margins of the vessel walls were everted with the aid of a specially designed forceps as the clips were being applied. Mal-aligned clips were likewise removed with forceps specifically designed for just such a purpose. Eight to 12 micro-clips were used for each connection to achieve water tight anastomosis.

In group I, the transected carotid arteries were anastomosed with continuous running 7-0 polypropylene sutures. Care was taken to ensure that only the needle came into direct contact with the arterial intima. In both groups, anastomosis was tensionless. Differences in bleeding, operative time, and surgical ease were carefully documented by one observer. The operative time was checked from the beginning of the first initiating suture or staple

until placement of the last suture or staple. All vascular connections were made by one surgeon.

### Angiographic preparation

Postoperative angiograms were performed in three animals. An overdose of KCl followed pretreatment with intravenous ketamine. All animals were fully heparinized. One animal was a 2 week survivor and the remaining two were 30 day survivors. Dye was injected retrogradely through an 18 gauge plastic needle tip placed in the proximal end of the transected descending aorta. After kilovolt peak and milliampere second settings, fluoroscopic guidance was utilized to obtain carotid arteriogram images on a standard A4 sized radiography film. The angiograms were assessed for stenosis and luminal shape.

### Gross, light and scanning electron microscopy (SEM)

Prior to specimen harvesting, heparinization (100 units per kg) was performed to prevent clotting. Ligatures were placed on the carotid artery about 1cm distally and proximally to the anastomosis site. The luminal aspect of the isolated carotid artery segment was then fixed under constant pressure with 4% formalin. The specimen was then harvested and prepared for hematoxylin and eosin (H&E) staining. Fibrin and thrombus formation, inflammatory cell infiltration in the adventitial and medial layers, foreign body reaction, endothelial disruption, luminal distortion, fibrosis, and intimal and medial thickening were examined under light microscopy (LM). One 24 hr specimen in which prolene was used on one carotid artery and VCS clips on the contra-lateral side was prepared for SEM. The methods have been described elsewhere (3). The differences in the suture line appearance, thrombus and or fibrin deposition, and the degree of mural penetration in each group were carefully recorded.

### Statistical analysis

Comparison of the two groups was made by simple non paired t-test where appropriate and assessment of the gross, microscopic and SEM findings were descriptive.

## RESULTS

### Surgical data

The differences in the outcome between the two groups are summarized in Table 1. The mean operative time was significantly reduced with the VCS clips, con-

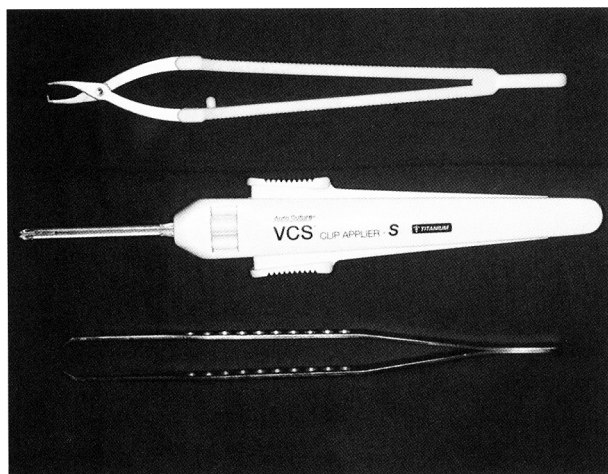


Fig. 1. The VCS clip applicator, forceps, and clip removers. The clips used in the current study are 0.9 mm small clips.

Table 1. Summary of the results between group I and II

|                              | Group I         | Group II          |          |
|------------------------------|-----------------|-------------------|----------|
| Operation                    |                 |                   |          |
| Operative time (min)         | 11±3.8          | 5±1.4             | (p=0.00) |
| Technical ease               | Moderate        | Markedly enhanced |          |
| Carotid angiogram            | Stenosis (>50%) | No stenosis       |          |
| Light microscopy             |                 |                   |          |
| Luminal contour              | Irregular       | Well preserved    |          |
| Thrombus                     | None            | None              |          |
| Fibrin deposition            | Moderate        | Mild              |          |
| Endothelial disruption       | Severe          | Mild              |          |
| Inflammatory infiltration    | Modreate        | Mild              |          |
| Giant cell formation         | Moderate        | None              |          |
| Scanning electron microscopy |                 |                   |          |
| Transmural penetration       | Yes             | No                |          |
| Endothelial disruption       | Yes             | No                |          |

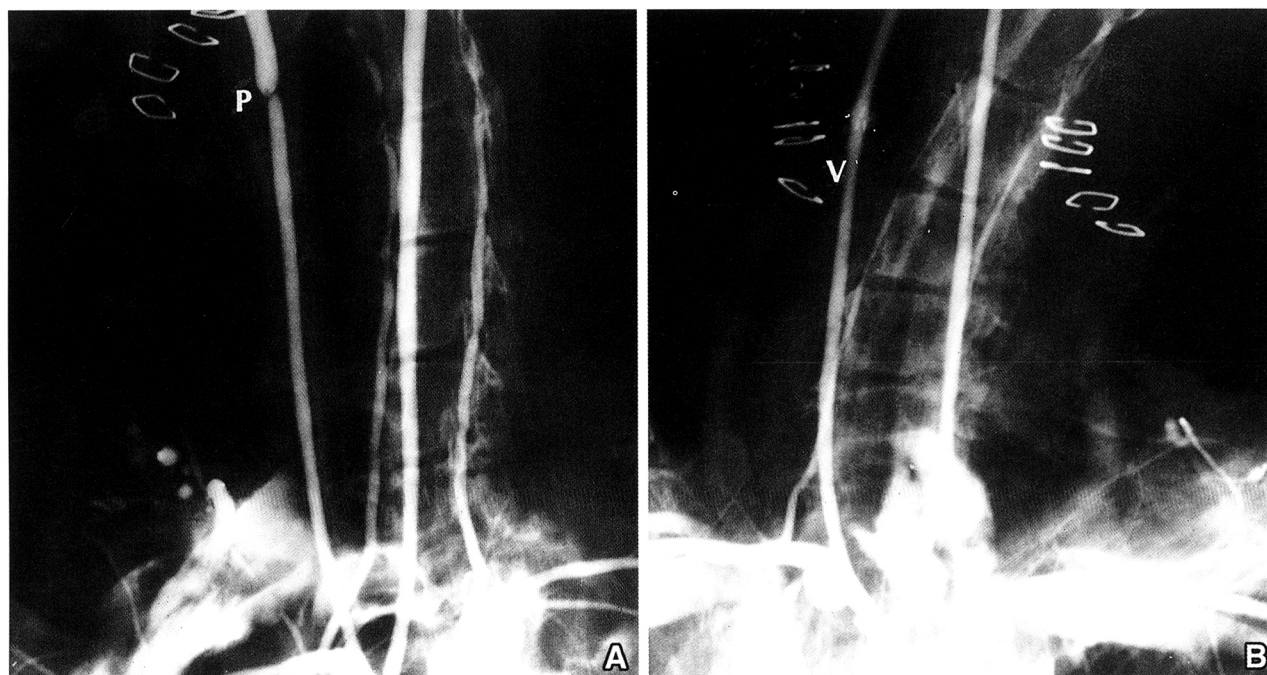


Fig. 2. Rabbit carotid angiograms obtained at 1 month postoperatively. In the polypropylene (P) specimen there is marked vascular stenosis. In the VCS (V) specimen, there is no signs of stenosis.

suming less than half the time with prolene suturing. Anastomosis using the VCS clips was technically easier compared to conventional suturing with 7-0 polypropylene sutures. The degree of bleeding was similar in both groups.

**Carotid arteriograms**

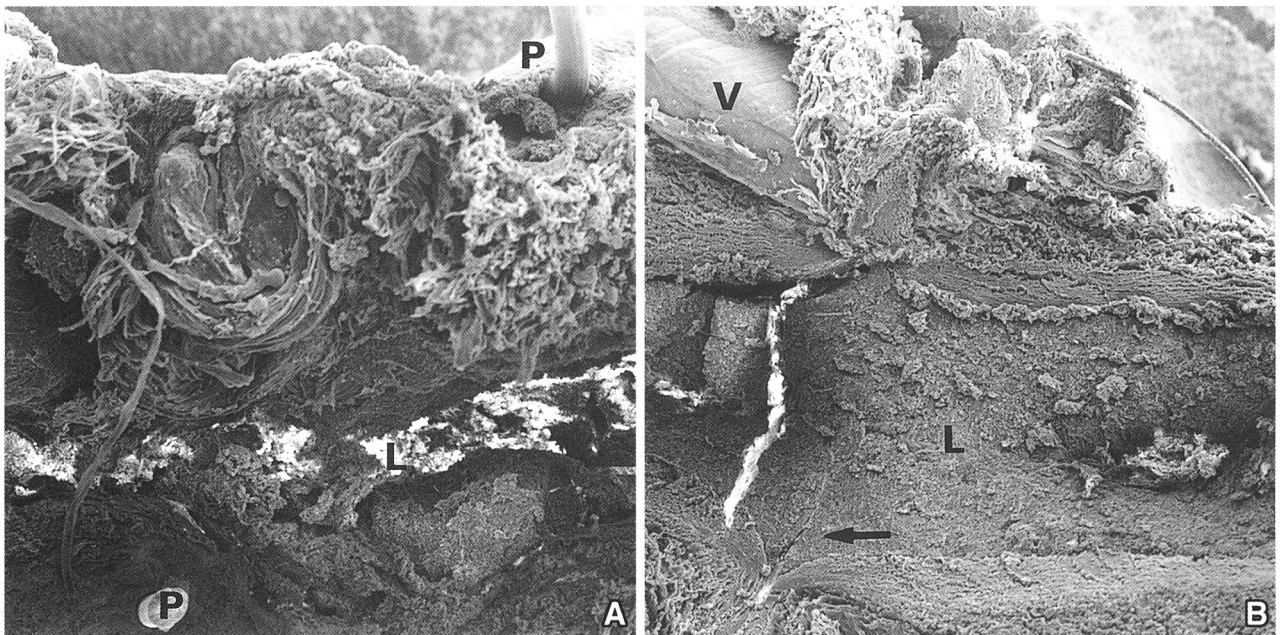
Carotid arteriograms at POD #14 and 30 days showed significant stenosis in group I in the anastomosis site whereas in group II there was almost no stenosis (Fig. 2).

**Gross and light microscopy**

The diameter of the normal rabbit carotid artery, as determined by passing small coronary dilators/probes ranged between 1.5 to 2.0 mm. Grossly, the luminal morphology was better preserved in group II than in group I. The lumen in group I had an irregular contour due to folds created by the sutures. Microscopically, differences in thrombus formation, fibrosis and intimal-medial thickening were minimal. However, fibrin deposition in group I was greater, especially at or near the suture sites. Traumatic injury was also greater in group



**Fig. 3.** Histologic section of the rabbit carotid artery at 14 days postoperatively. In the group I specimen (A), degeneration of the arterial wall and distortion of luminal morphology is evident (arrows) whereas in the group II specimen (B), minimal luminal disruption and a well preserved lumen is present. Note the VCS staples penetrating the vascular wall in a non-transmural fashion (arrow).



**Fig. 4.** 24 hr SEM specimen shows irregularity and luminal narrowing with abundant fibrin build up in the group I specimen (A), whereas in the VCS specimen (B), the lumen is widely patent with only a thin crease like suture line (arrow). P, prolene; V, VCS; L, lumen.

As shown by the degenerative changes and endothelial disruption (Fig. 3). In group I fibrin deposition was present in both intima and adventitia. The adventitial and medial inflammatory cell infiltration were moderate in group I whereas inflammatory infiltration was minimal or nil in group II. Furthermore, multinucleated giant

cells in the vessel walls were present only in group I, while, the lumen was well preserved in group II.

#### Scanning electron microscopy

In group II the approximated vessel margins on scan-

ning electron microscopy were everted with avoidance of full thickness penetration. As a result, a smooth continuous luminal surface was preserved. In group I, the entire thickness of the vessel wall was penetrated with luminal surface disruption (Fig. 4). In group II, the approximated edges were evident by only a barely visible linear crease-like line. There was minimal fibrin or thrombi deposition. No clips protruded through the lumen.

## DISCUSSION

The vascular clip system clip applicator allows surgical ease and expedience through automation. Vascular injury is minimized with non transmurally penetrating sutures. Clinical restenosis in the first several months after vascular reconstruction is mostly attributable to intimal hyperplasia. This is mostly the result of wound healing, which is a universal response elicited by physical injury to tissue, including vessels. It is characterized by proliferation of smooth muscle and myofibroblastic cells. Interstitial matrix formation is increased by the deposition of collagen and various ground substances leading to intimo-medial thickening (4, 5). The major advantages of the vascular clip system, which is a relatively novel suturing device, in theory, lie in its ability to effectively preserve the endothelial lining and avoid intraluminal exposure to foreign materials (6). Intermediate term saphenous vein graft failure, which comprise 15-20% of all coronary artery bypass graft failures (7), generally occur within the first 2 yr of surgery, most likely from endothelial injury and/or dysfunction (8). Conventional suturing with polypropylene may cause mechanical injury to the endothelium triggering the release of various growth factors and cytokines such as platelet derived growth factor (PDGF) (9) or transforming growth factor-beta 1 (TGF beta-1) (10). A series of biological responses are then activated leading to variable degrees of neointimal proliferation and luminal narrowing. The non-penetrating nature of vascular anastomosis with the VCS clips causes significantly less endothelial injury. Indeed, comparison of the two study groups showed a clear disparity in the histologic findings regarding fibrin deposition, inflammatory cell infiltration, multinucleated giant cell formation, endothelial disruption, and luminal distortion where these changes were more prominent in group I than II. The inflammatory cellular infiltrates comprised mostly lymphocytes and abundant macrophages. Signs of vascular degeneration and transmural injury were also prominent in group I, especially, near the sites of suture penetration. The SEM images of the VCS specimen showed a well preserved luminal surface and eversion of the stapled resected vascular margins. The approximated sutured

edges remained as a barely noticeable linear crease. Preservation of the endothelial lining has the advantage of providing a thrombo-resistant luminal surface which inhibits platelet activation (11) and subsequent release of cellular cytokines and growth factors. Thus, by preserving the integrity of the endothelial lining, the VCS clips may lead to superior graft patency. With the VCS clips, preservation of a smooth uninterrupted endothelial surface may be achieved through proper tissue everting techniques. The clips must be placed sufficiently close together (about 1 mm apart) to secure a water tight hemostatic suture lining. As application of additional clips may not only be difficult but also potentially traumatic, each clip must be placed carefully and properly the first time (6).

The VCS clip applicator, in many instances, showed the ability to significantly reduce the operative time compared to conventional methods (3, 6, 12). In the present study, a striking reduction in the operative time was demonstrated with the VCS clips as it was reduced by more than half the time required with polypropylene.

Angiography showed severe stenosis with polypropylene as opposed to the VCS clips which showed almost no or minimal stenosis, suggesting a potential for superior flow dynamics and perfusion in the latter. Davies et al. (7) noted that vascular narrowing with polypropylene may result in poor proximal flow and graft occlusion with early graft failure occurring within the first 30 days.

Despite the contrasting nature of the findings between the two groups in the current study, the small number of animals and relatively short study period were major shortcomings. Furthermore, the study was conducted on an experimental animal model, drawing argument over the question of clinical applicability. In the clinical situation, certain angles may be very difficult to approach, with the potential for increased time consumption, compromise of graft patency, poor hemostasis and trauma. However, the efficacy and technical feasibility of this system were readily demonstrated in other well conducted clinical trials (6) and in so far as this was mainly a feasibility study, the aims of the current study were fulfilled. Regardless of the small number of animals, the differences in the findings between the two groups were clear. Therefore, a larger animal group was not necessary.

One potential flaw in the experimental design was in the different suturing techniques used in the two groups; In group I, suturing was continuous whereas in group II it was interrupted. This was not a biased effort to ensure a larger diameter in the VCS group, but to simulate the actual clinical setting where coronary artery bypass grafting is performed with running continuous sutures. By the same token, coronary artery bypass grafting using the VCS clips would be achieved with multiple

interrupted application of clips.

The documentation of bleeding amount, operation time, and ease of surgery may vary among surgeons. In order to eliminate this individual variation, one surgeon performed surgery throughout the study, and operative data regarding these parameters were also recorded by one person.

The pathologic findings in both groups were derived from the observation of a single observer. Due to the subjective nature of the pathologic findings, the pathologic data was mainly descriptive. However, the aim was to assess for any differences between the two groups. The data being derived from a single observer showed a difference in trend between the two groups.

In conclusion, the VCS clip applier was superior to suturing with polypropylene in the following areas: 1) preserving luminal endothelial lining, 2) decreasing fibrin deposition and foreign body reaction 3) significantly reducing the operative time, 4) enhancing technical ease, and 5) preserving luminal morphology more effectively and causing less stenosis. With further research, the VCS clip applier may present as a viable option in vascular anastomosis of fine caliber vessels such as coronary arteries.

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