Endothelialization after arterial and venous micro-anastomosis

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Knowledge of the initial time required to repair the endothelial surface of small vessels after microsurgical vascular anastomosis of veins and arteries is required to determine the preferable duration of antiplatelet prophylaxis and anticoagulation after emergency or elective microsurgery. To determine this, the femoral arteries and veins of 16 Sprague-Dawley rats were isolated, sectioned and repaired with microsurgical technique. The animals were then killed at one day intervals from the first to the 16th postoperative day. Femoral veins and arteries were harvested, sectioned and prepared for scanning electron microscopy. The results show that endothelialization of the repair line is begun by day 3 and completed by day 7 in the veins and arteries. Endothelialization of the intraluminal protruding sutures takes nine days in the veins while it is only starting at day 15 in the arteries. If this model can be extended to the human clinical situation, antiplatelet prophylaxis or anticoagulation should be administered for at least seven days. Further study is required to evaluate the thrombogenic potential of intraluminal protruding sutures.

Key words*: Anti-platelet therapy, Endothelial trauma, Endothelialization, Microsurgery, Prophylactic anticoagulation, Vascular anastomosis*

Endothélialisation après micro-anastomose artérielle et veineuse

RÉSUMÉ : La connaissance du temps initial nécessaire pour réparer la surface endothéliale des petits vaisseaux après une anastomose vasculaire microchirurgicale de la veine et des artères est nécessaire afin de déterminer la durée préférable de la prophylaxie antiplaquettaire et de l'anticoagulation après une microchirurgie urgente ou non. Pour mesurer le phénomène, les fartères et veines fémorales de 16 rats Sprague-Dawley ont été isolées, sectionnées et réparées par technique microchirurgicale. Les animaux ont par la suite été euthanasiés à un jour d'intervalle, du jour 1 au jour 16 postopératoire. Les veines et les artères fémorales ont été mises en culture, sectionnées et préparées en vue d'une microscopie électronique par balayage. Les résultats démontrent que l'endothélialisation de la ligne de réparation commence dès le jour 3 et est achevée dès le jour 7 au niveau des veines et des artères. L'endothélialisation des sutures intraluminales prend neuf jours dans les veines et ne commence qu'au jour 15 dans les artères. Si ce modèle peut être

extrapolé pour s'appliquer à une situation clinique chez l'humain, la prophylaxie antiplaquettaire ou l'anticoagulation devrait être administrée au moins sept jours durant. D'autres études sont nécessaires pour évaluer le potentiel thrombogène des sutures intraluminales.

In clinical microsurgery involving vessel anastomosis for elective reconstruction or emergency replantation, thrombosis of the vascular repair is the most feared complication. Trauma to the endothelium is known to be a major thrombogenic cause because of the exposed vessel wall. Therefore, to decrease the risk of thrombosis at the site of anastomosis, antiplatelet agents or anticoagulation has been advised by some for a duration varying from five days to two weeks. The endpoint of therapy is the time for re-endothelialization of the blood vessel which has been noted to vary from seven days to four weeks by different authors (1-6). Since complications from antiplatelet and anticoagulation therapy exist in terms of bleeding, hematoma, allergic reaction, and that the associated cost of those therapies can be significant, it is important to find out more precisely how long the duration of endothelialization is in small veins and arteries in order to determine better when to stop the prophylactic therapy safely.

MATERIALS AND METHODS

The study was started after approval by the Committee for Humane Use of Animals of SUNY Health Science Center at Syracuse, New York. Sixteen male Sprague-Dawley rats weighing 210 to 325 g were selected for the experiment because their femoral arteries and veins had diameters ranging from 0.5 to 1.1 mm. The rats were anaesthetized with intraperitoneal nembutal 6.5 mg/100 g of body weight and supplemented as needed during the procedure. Under sterile conditions through a transverse inguinal incision, the femoral arteries and veins were carefully exposed, mobilized between the inguinal ligament and the inferior epigastric vessel and divided. The microsurgery anastomoses were performed under double Acland clamps using interrupted 10-0 nylon sutures (0.2 metric, BV75-3 needle, Ethicon). The number of sutures varied from six to 10 per vessel. The double clamp was released. The artery was first repaired and then the vein. The wound was closed and the contralateral side was also operated on with the same technique. Postoperatively, the rats received torbugesic subcutaneously if needed for pain. They were maintained in separate cages with infrared heating lamps for the first few postoperative days. The animals were killed at one day intervals after surgery starting on the first day through to the 16th day. At the time of sacrifice, nembutal intraperitoneal anaesthesia was given. Sutures were removed and the wound opened. Dissection was carried down to the femoral vessels which were identified and mobilized. Each vessel was harvested, opened longitudinally and irrigated with lactated Ringer's solution. Specimens for electron microscopy were then immersed in a 2.5% buffered glutaraldehyde solution. The animals were then killed with an overdose of intraperitoneal sodium pentobarbital according to the American Veterinary Medical Association report on euthanasia (1993).

Preparation for scanning electron microscopy

The specimens were harvested and immersed in 2.5% glutaraldehyde 0.1 M phosphate buffer solution for 24 h for fixation. They were rinsed in 0.1 M phosphate buffer, fixed in 2% osmium tetraoxide for 1h and then rinsed again in 0.1M phosphate buffer. They were dehydrated in ascending concentrations of alcohol and with Peldri II as an alternative to critical point drying (7). The specimens were then coated with carbon and gold and then kept in a vacuum chamber.

Scanning electron microscopy examination was performed using Hitachi S520 SEM at an accelerating voltage of 20 kV. Images were then photographed using Polaroid film.

RESULTS

At one day intervals from the first to the 16th postoperative days, the right and left femoral arteries and veins were isolated, their patency was evaluated and they were then harvested. Fifty-three out of 64 vessels were patent for an overall patency rate of 82.8%. Twenty-five out of 32 arteries were patent for a patency rate of 78.1%. Twenty-eight out of the 32 veins were patent for a patency rate of 87.5%.

The operative time for the four vessel anastomoses ranged from 67 to 127 mins, for a mean of 95 mins. All rats gained weight from the day of surgery to the sacrifice day except for the rats that were killed on days 1 and 2, which did not gain weight, and the rat that was sacrificed on day 3, which lost 7 g. All of the other rats gained from 17 to 90 g between the day of surgery and the day of sacrifice.

Microscopic findings

At the time of harvesting after dividing the vessel and gently irrigating the lumen with lactated Ringer's solution, the presence of a thrombus, the quality of the endothelial lining, the visibility of the threads, and the overall appearance of the inside of the vessel were evaluated. When the vessel was patent, the luminal wall always appeared shiny and smooth. With thrombosed vessels, the lining of the lumen appeared scaly. Up until day 4, small thrombi were present around the sutures in both veins and arteries. Starting on day 8, the sutures appeared to be covered in the venous specimen while they were visible in the arteries up until day 16.

Scanning electron microscopy

Day 1: The anastomosis line is visible in both the venous and the arterial repair. Platelets, aggregates and thrombi are visible, covering the suture line in the vein, whereas the sutures are very visible and protruding within the lumen of the artery, having only the entry and the exit wound of the endothelium covered with platelets and red cells (Figure 1).

Day 2: The repair line is visible in both the artery and the vein. It is covered by thicker, more significant aggregate of platelets and red blood cells in the vein. In the vein, the sutures are covered by a fibrinous clot. In the artery, sutures are much more visible.

Day 3: The repair line is still covered by fibrinous clot in the vein. Part of the sutures are now visible. The clot is thinner. The artery repair line is covered by spindle shaped cells scattered transversely and longitudinally, as opposed to the constant longitudinal

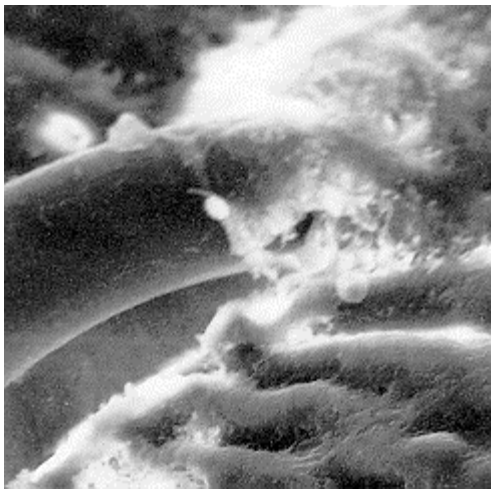

Figure 1) *Day 1: Scanning electron microphotograph showing platelet and red blood cell aggregate. Suture enters the vessel wall (X 1000 magnification)*

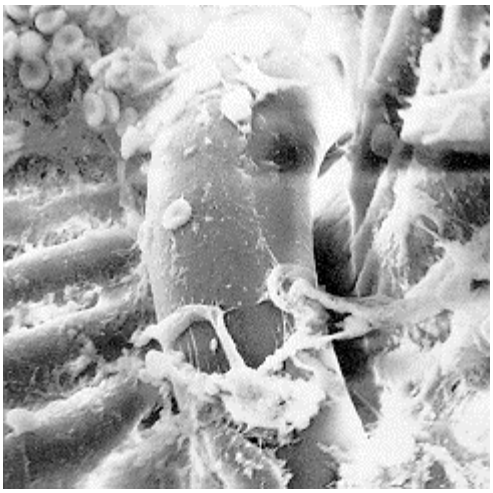

Figure 2) *Day 3: Electron microphotograph of the artery showing the suture coming in the vessel lumen, multiple red cell aggregates, and the fibrinous layer on the thread (X 1000 magnification)*

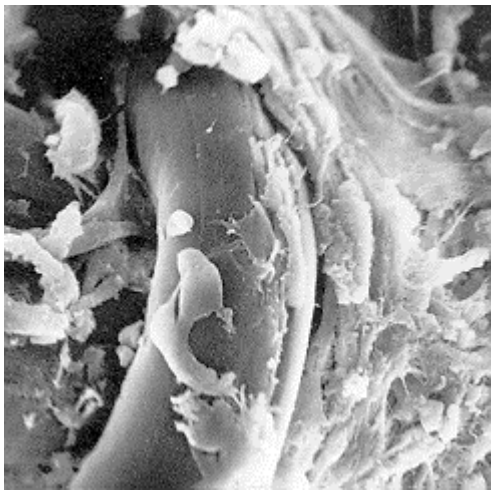

Figure 3) *Day 6: Scanning electron microphotograph of the vein showing the suture, fibrinous clot, stellate cells on the suture and a large artifact (X1000 magnification)*

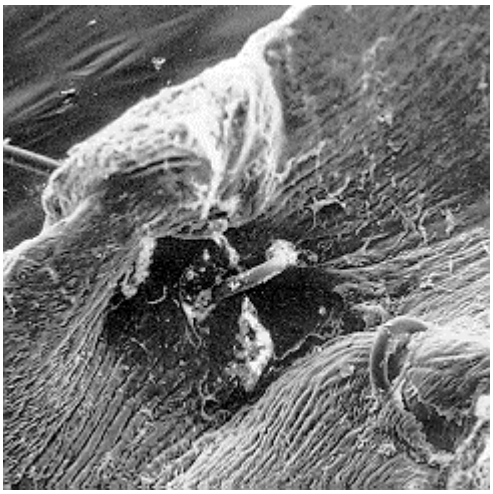

Figure 4) *Day 8: Scanning electron microphotograph showing the protruding suture in the lumen of the artery at the site of the repair. Note the longitudinally arranged endothelial cells at the repair site (X 100 magnification)*

organization in the remainder of the vessel. Red blood cell aggregates, fibrinous material, and very flimsy layers cover part of the threads (Figure 2).

Day 4: Suture line in the vein is still covered with fibrinous clot. In the artery, sutures are still visible in the lumen. Repair line is not visible per se but the folding caused by the suture shows the level of the repair. Thin endothelial cells cover the repair line.

Day 5: The fibrinous clot covering the venous repair site is much thinner and smaller, revealing some of the sutures protruding into the lumen. The repair line itself is not visible in the vein. In the artery, the repair line is not visible but sutures are protruding in the lumen.
Day 6: The venous repair line is not visible. The intraluminal suture in the vein starts to be covered by flat endothelial cells (Figure 3). The arterial repair line is not visible per se. The sutures are covered by a thin fibrinous clot.

Figure 5) *Day 10: Scanning electron microphotograph of the vein showing the completely covered suture slightly bulging into the lumen (X 1000 magnification)*

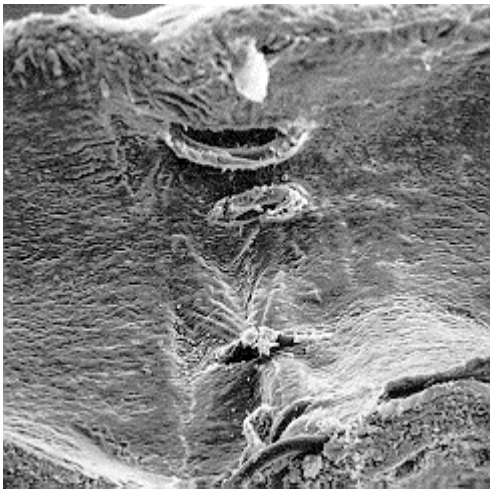

Figure 6) *Day 12: Scanning electron microphotograph with the back scattered electron detector showing the site of the arterial repair with the partly covered suture (X 100 magnification)*

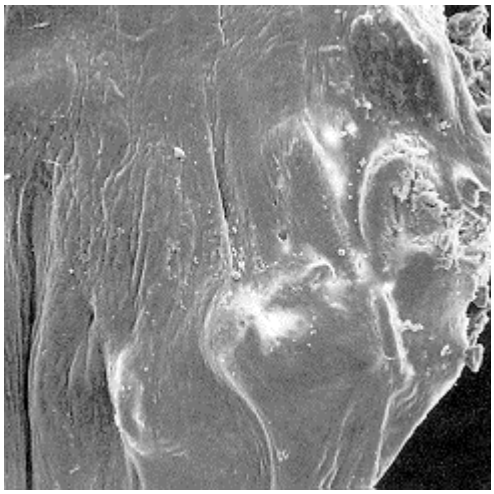

Figure 7) *Day 13: Scanning electron microphotograph of the repair site of the vein showing the covered sutures. Note that the repair line is not visible (X 100 magnification)*

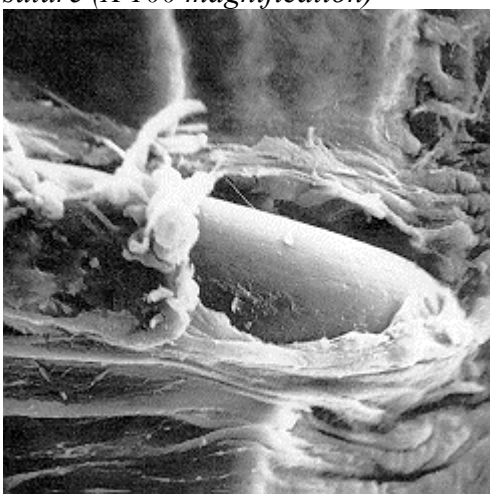

Figure 8) *Day 16: Scanning electron microphotograph showing a part of a suture being covered by some endothelial cells in the artery (X 1000 magnification)*

Day 7: The repair line of the vein is not visible and is covered by longitudinally oriented endothelial cells. The arterial repair is also covered by longitudinally oriented endothelial cells.
Day 8: Venous repair site completely covered by endothelial cells. It is identified by the slight bulge made by the covered sutures. Arterial repair line is not visible. The sutures are still protruding within the lumen (Figure 4).
Day 9: Venous repair site is completely covered by endothelial cells. The arterial repair site is also covered by endothelial cells. The suture is completely visible within the lumen of the artery and there is a decrease in the amount of the fibrinous clot at the base of the suture at the entry site of the needle.
Day 10: In the vein, the sutures are completely covered and only a slight bulge is a sign of the repair site. The artery still shows the bare sutures (Figure 5).
Day 11: The venous specimen is similar to day 10. Few red blood cells are seen attached to the bulge made by the covered suture. In the artery the sutures are partly covered by red cell aggregate.
Day 12: Findings in the vein are similar to days 10 and 11. Arterial repair line is not visible and the sutures are partly covered by fibrinous material (Figure 6).
Day 13: The venous repair is completely covered. The inside of the vessel is smooth. The sutures can be imagined under the deformity, otherwise smooth endothelium. In the artery, except for the visible sutures, the repair line is not recognizable (Figure 7).
Day 14: The repair site in the vein is still very smooth with shadows created by the deformity from the underlying sutures. The sutures are visible in the arterial lumen. The suture enters the lumen and a cuff of endothelial cells appears to be extending onto the suture.
Day 15: Venous repair site is not visible anymore except for slight bulges created by the underlying sutures. The arterial repair site is not visible except for partially protruding sutures which appear to start to be covered by endothelial cells.
Day 16: Similar findings in the vein from the previous days. At the site of arterial repair, sutures appear to be more covered by irregular endothelial cells (Figure 8).

DISCUSSION

The repair of anastomotic sites is a complex sequence of events involving hemostasis and repair of the vessel wall. The process is slightly different in the vein than in the artery. It appears that although the hemostatic event of red cell, platelet depositions, formation of fibrinous clot in the entrance and exit wounds of the sutures are similar in the veins and the arteries, the process nevertheless differs slightly between the two vessels. Endothelialization of the repair line in between the two cut edges of vessels seems to occur in both vessels around day 3. After the repair line is covered with endothelial cells, they are later reorganized and realign longitudinally as in the remaining vessels. Thereafter, endothelial cells creep onto the sutures in the venous anastomosis, starting at day 6 to cover the sutures completely at day 9. On day 7, the repair lines are completely covered both in the artery and the veins and the endothelial cells are aligned in a longitudinal direction. In the arterial repairs, the sutures are not covered by endothelial cells or even fibrinous clot as in the veins for the first two weeks. A beginning of cuffing of endothelial cells seems to start around day 15 and 16 to cover the proximal and distal parts of the intraluminal suture.

In cases of clinical microsurgery, the risk of thrombosis of the anastomosis site is considered to be significant. The antiplatelet or anticoagulation prophylaxis duration of treatment should therefore vary for protection of the venous or arterial anastomosis. In both cases the repair lines seem to start to be covered by day 3 and are completely covered by endothelial cells by day 7. The intraluminal sutures are covered in the vein by the ninth postoperative day while in the artery they are incompletely covered by day 16. If the rat model can be extended to the human situation, this study therefore supports the proposal that prophylaxis should be given at least until the end of the first week to protect the thrombogenic effect due to the repair line itself. These findings might explain the clinical situations where heparinization is given for five days, then discontinued, and cases of replant failure have occurred thereafter.

The question of the thrombogenicity of the exposed intraluminal suture still remains. In the event that it would be significant, venous repair should require prophylaxis for nine to 10 days while arterial repair would require much longer prophylaxis. Further work needs to be done on this question.

Although this study uses a rat model that cannot necessarily be extended to the human situation, it identifies a slightly different process of endothelialization in the arterial and venous injury and repair. The complete endothelialization of exposed intraluminal material takes a longer time in the artery than in the vein.

CONCLUSION

Endothelialization of the repair line in the rat femoral artery and vein is completed by day 7. The complete endothelialization of intraluminal suture is faster in the vein, being completed at nine days, while it takes more than 16 days in the artery. Since intraluminal monofilament sutures are probably of low thrombogenic potential, antiplatelet therapy or anticoagulants should be administered for at least one week postoperatively. Further study is required to evaluate the actual thrombogenic potential of intraluminal suture material.

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