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# A SCANNING ELECTRON MICROSCOPIC STUDY OF END-IN-END AND END-TO-END MICROVASCULAR ANASTOMOSIS IN THE RAT FEMORAL ARTERY

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*The occurrence of thrombotic occlusion & endothelial injuries at the site of anastomosis have been considered as major problems in microvascular surgery.*

*The purpose of this study was to ascertain whether a end-in-end(sleeve, telescope) anastomosis compared favorably with end-to-end anastomosis in healing procedures on the endothelium and to study the possibility of clinical application in end-in-end method.*

*The microvascular anastomoses have been performed with end-in-end method in the femoral arteries of 20 rats group, also with end-to-end method on the same arteries of another 20 rats group, and then the four anastomosed vessels in subdivided groups on each group were taken after period of 1, 3, 7, 14, 21 days following by anastomoses for scanning electron microscope observation.*

*The results were as following:*

1. *The patency rate was 90% in the end-in-end group and 85%in the end-to-end group and late thrombus occurred at 7, 14 days on both groups, which suggested —consistent monitoring of patency was required for two weeks at least.*
2. *Platelet aggregates at the site of anastomosis began to organize on post-operation 3 days and in the end-in-end group, the initially decreased lumen of inserted vessel was gradually increasing on 7 days due to atrophy of the medial layers.*
3. *Re-endothelialization was completed between 7 and 14 days in end-in-end group, whereas between 14 and 21 days in end-to-end group.*

## I. INTRODUCTION

The level of microvascular surgery has reached to the general application method after being achieved with introduction of microscope by Nylen in 1921. Nowadays, the clinical value appears every specialized department of reconstructive surgery including free composite tissue transfer which involves the bone, muscles, nerves, vessels and skin[1].

There were, in many instances, applications of free autogenous bone grafting in trauma and ablative cases but the problems of bone resorption occurred in the various kinds of patients of every condition, age and recipient tissue bed.

But clinical problems previously deemed insoluble have been successfully treated by the application of the microvascular surgery.

The success rate of vascular surgery depends on the patency of the vessel. Numerous affecting factors in the patency rate are the diameter of vessel, the distance of the end of vessels, the injured state of tissue, the technique of vascular surgery.

The most common cause is technical error in the suturing of the vessel. It can be the injuring cause of intima of vessel which stimulates thrombus formation, as the result, the failure of operation can be occurred. Howerver, even the most technically perfect anastomosis cannot eliminate the damage which microneedles cause to the vascular endothelium nor eliminate foreign body reaction to the holes in the lining endothelium.

Lauritzen[2], in 1978, adapted the vascular invagination technique to microvascular anastomosis using only two sutures, the end of vessel sleeved into the other end of vessel lumen only suturing of media, adventitia of vessels. The end-in-end(invagination) technique has been compared with the end-to-end technique as to thrombus formation and vascular tissue repair by other scholar's following reports.[3,4,5,6,7,8].

This author attempted to ascertain whether a end-in-end(EIE, invagination, sleeve, telescope) anastomosis compared favorably with end-to-end(ETE) anastomosis in healing procedure on the endothelium and to study the possibility of clinical application in EIE method.

The microvascular anastomosis has been performed with EIE method on the femoral arteris of 20 rats group, also with ETE method on the same arteries of another 20 rats group, and then the four anastomosed vessels in 5 subdivided group on each group were taken after periood of 1, 3, 7, 14, 21 days following by anastomoses for scanning electron microscopic observation.

## II. MATERIAL & METHOD

### 1. Material

40 male white rats weighing 250-350gm(Sprague-Dawley), a basic microvascular set: a microscissors, needle holder(Keeler Co.), atraumatic clamps, Acland approximator

operative microscope: stereoscope(American Optical Co.)

suture material: BV 130-0, 10-0 Ethilon Inc. supplies

applied vessel: the femoral artery of the rat(0.8-1.0㎜ diameter)

### 2. Preparation & Method

(1) Preparation

Induction anesthesia was accomplished using Ether and then Ketamine Hydrochloride(Ketalar) was injected peritoneally a dose of 0.1㎎/gm(10㎎/㎖) for maintenance of anesthesia.

The rat was placed to the supine position on the dissecting board and secured its legs with rubber bands.

Using scissors, an incision along the entire length of the inguinal fold as 3㎝ in length exposing the inguinal fat pad was made.

The pad was retracted laterally to expose the femoral triangle and vessels.

When the femoral vessel bundles was seen, the overlying bundle fascia could be separated from the adjacent tissue.

When freeing up the femoral artery, 2% lidocaine was flooded to relieve the spasm for 5 minutes.

Under 16×magification, vessel approximating clamp was applied to the artery as far distance as possible between the clamp jaws. Transection of the artery with microscissor & irrigation of the lumen with heparinized saline(1,000μ/100㎖)was done.

(2) Method

End-In-End Anastomosis

The adventitia on excised vessel ends was removed as 2㎜ in length. After the distance between Acland clamp jaws was approximated the suture needle on the distal end of the vessel was fully inserted on the entire layer of the endothelium and on the proximal end, was partially penetrated on the medial, adventitial layers of the endothelium at the site 2㎜ departed from the end of the vessel(2 sutures, at 60 degrees away).

The clamp was turned over, and the drooped pro-

ximal end of the vessel was pushed into the distal end of the vessel with a pair of No.5 jeweler's forceps.

The opposite surface was also anastomosed as previous described procedures.

This end-in-end anastomosis technique is the Nakayama's modified invagination technique(Fig. 1)

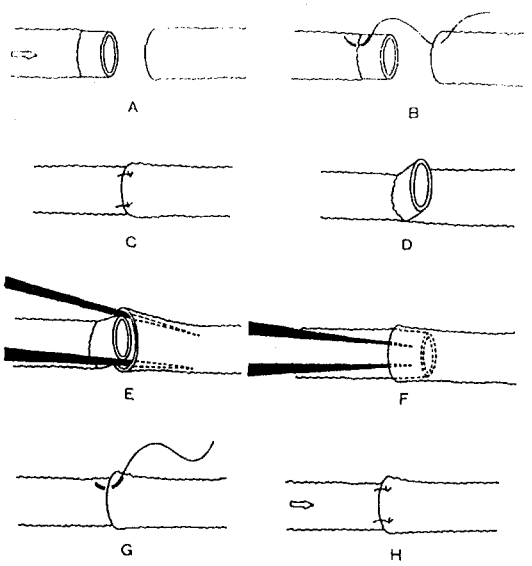


Figure 1.
- Nakayama's end-in-end anastomosis
A. Minimal stripping of the adventitia
B. Taking a bit of adventitial tissues only, in the up-stream segment
C. Two sutures are placed 60° apart and tied
D. Posteriorly, the up-stream segment is bent and protrudes.
E. Down-stream segment is dilated
F. Up-stream segment is inserted
G. One or two sutures are added
H. The anastomosis is checked to ensure that the invaginated portion has been inserted evenly.

End-To-End Anastomosis

After removal of adventitia on vessel ends, the triangular technique using of 3 guide sutures(at 120 degrees apart) was first placed and added by 3-5 interrupted sutures according to the vessel size.

under the microscopic visualization, the distal clamp was released and the quality of back-flow assessed. the proximal clamp was then removed. A patency test was done at 20 minutes to confirm the status of the anastomosis(Fig. 2).

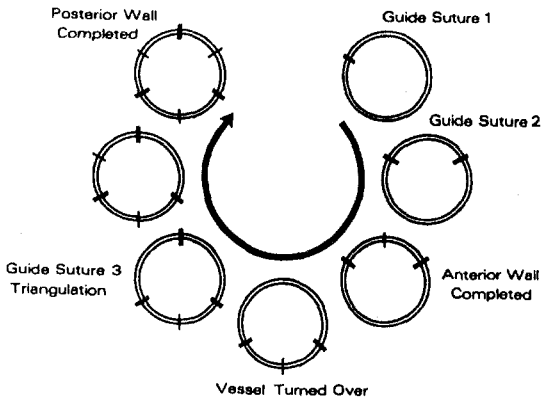


Figure 2.
- A-six-suture end-to-end anastomosis
: It consists of three guide sutures and three interrupted sutures.
With increasing diameter, an additional number of interrupted sutures would be placed in between the three guide sutures.

(3) Tissue Specimen preparation

After obtained from the vessel in the period of postoperation 1, 3, 7, 14, 21 days tissue specimen was excised longitudinally, then fixed in the mixture of 2% paraformaldehyde and 2.5% glutaraldehyde(0.1 M phosphate buffer, PH 7.4) for 12 hours at the 4℃ and washed 3 times for 5 minutes.

The specimen was repeatedly fixed in the 1% Osmic acid(OsO4) at the room temperature for an hour and washed 3 times for 5 minutes in the 0.1M phosphate buffer solution.

The fixed specimen was dehydrated in the graded ethanol(50%, 70%, 80%, 90%, 100%), carried into the acetone and dried up by Critical Point Dryer, Co2 gas.

Finally after coated with spurted gold at the Ladd Bench Top sputter Coater, the prepared specimen

was observed under Scanning Electron Microscope (Nalolab 2100).

We also prepared the specimen from the normal vessel to compare with operation group.

## III. RESULTS

### 1. Patency Rate

In the End-In-End group, the thrombus was noticed on postoperation first day and on seven day.

The result showed patency rate of 90% (18 of 20).

In the End-To-End group, the early thrombus formation was discovered with 3 day, the late thrombus formation with 7 day and 14 day.

The result of the rate was accepted as 85% (17 of 20) (Table 1).

### 2. Scanning Electron Microscopic Findings

(1) non-operated control vessel group

Endothelial cells showed round or rhomboid-shaped cells, arrangement of paralleling to the blood stream.

The microvilli scattered on its luminal surface with longitudinal ridges. mechanical injury sites also can be seen(Photomicrograph 1,2).

(2) end-in-end anastomosis

The jeweler's forceps was used to invaginate the proximal end of the vessel into the distal end, as the result of invagination, endothelium was partially injured & noticed with platelet aggregates to the site (Photo. 3,4, 1 day after E-I-E anastomosis).

Macrophages, monocytes, desquamated cells in the lumen was observed and the cell group with platelet aggregates organized(photo. 5,6,3 day after E-I-E anastomosis).

Reendothelialization has occurred on the proximal end of the vessel and made the longitudinal ridges of internal elastic lamina.

Atrophy of medial layer of inserted vessel was seen.

Flat shaped-neointima cell group can be observed in the lumen(Photo. 7,8, 1 week afte E-I-E anastomosis).

Neointimal layer of vessel had made of continuity of vessel ends. The internal diameter at the anastomosed site of the vessel was wider than that before days. Hyperplasia of medial layer also was seen (Photo. 9,10, 2 weeks after E-I-E anastomosis).

Thinning of vessel walls made inserted site indiscernable from the normal site(Photo. 11,12, 3 weeks after E-I-E anastomosis).

(3) end-to-end anastomosis

Mural thrombus which consists of platelets, RBC, tissue fibers has been noticed at the sutured area (Photo. 13, 1 day after E-T-E anastomosis). Desquamated endothelial area was found & thrombus

Table 1. patency rate according to anastomotic methods

| Postanastomotic interval | end-in-end | | end-to-end | |
|---|---|---|---|---|
| | P | T | P | T |
| short term group | | | | |
| 1 day | 3 | 1 | 4 | 0 |
| 3 days | 4 | 0 | 3 | 1 |
| Long term group | | | | |
| 1 week | 3 | 1 | 3 | 1 |
| 2 weeks | 4 | 0 | 3 | 1 |
| 3 weeks | 4 | 0 | 4 | 0 |
| Total | 18 | 2 | 17 | 3 |

(P ; patent, T ; thrombosis)

still was seen(Photo. 14, 3 day after E-T-E anastomosis).

Mural thrombus disappered and re-endothelialization was begun(Photo. 15, 1 week after E-T-E anastomosis).

Suture thread was covered with neo-intimal layer (Photo. 16, 2 week after E-T-E anastomosis).

Complete healing of endothelium was identified as smooth luminal surface, longitudinal ridges. Monocyte-like cells was also seen(Photo. 17, 18, 3 week after E-T-E anastomosis).

## Ⅳ. DISCUSSION

The microvascular surgical technique have been developed very much in recent decades owing to the dedicated, innovative microsurgeon, materials.

In 1590, Ambroise Pare introduced the ligature to surgery for controlling arterial hemorrhage but couldn't restore the anastomical continuity between the ends of a severed vessel.

In 1901, Payr devised "anastomotic tube" that were inserted into the lumens and the vessel ends sutured.

Followed by Carrel, in 1902, developed a continuous over-and-over suture technique in anastomosing blood vessels[1,9].

The Carrel's technique had become well known to the surgeon the world over but because of the risk of infection, it couldn't be immediately applied clinically.

However, Jahnke and Sleeley improved the patency rate with rapi suturing procedure, insertion of veind graft as needded, continuous everting mattress sutures at the beginning of the Korea War and reported extremities amputation rates of only 8% as being compared with an amputation rate of 50% followed by simple ligation.

The extent of vascular surgery rapidly expanded toward the periphery vessels but encountered the difficulty in small arteries in the 2.0 to 4.0㎜ range. In 1960, Jacobson and Suarez reported a 100% success rate for 1.4㎜ arteries by the introduction of new microscope and microinstruments and microsutures needles.

The advancement of microvascular research has suggested the possibilities of the anastomosis of 0.5㎜ vessels.

But the incidence of vascular thrombus formation has appeared when very small vessels were anastomosed[1,10,11].

There have been some reports concerned about microsuture techniques which could reduce the injuries of vascular endothelium[12,13,14].

Lauritzen[2](1978) reported that simple new technique using two sutures not penetrated the intima of vessel, invaginated proximal end into distal end of vessel could be compared similarity to the traditional end-to-end technique on the patency rate.

The advantage of the technique, what is called as invagination, sleeve, end-in-end anastomosis, has been advocated by the short operation time, simple technique, less injury condition.

Thereafter, the application of the various experimental & clinical part in reconstructive microosurgery has been testified by the authors[7,8,15]. But because of the stenosis & thrombus formation, its value in the empirical use still has remained to be discussed.

Although the hesitation was encountered in clinical application, the apprehension was reversed when Nakayama[16](1987) reported the successful results of 15 free flap transfer cases.

In a sequential paper submitted with using the new microvascular techniques, Lauritzen[2](1978) presented a 90% success rate in femoral artery and vein of 43 rats.

Krag and Holck et al[17,18,19](1980) et al also reported high patency rate of the telescopic technique in rats compared to the end-to-end method.

In Korea, the experimental researches were reported by Dr. kim[20,21](1980, 1983), Yu[22](1983). The authors' results were very successful as 84 to 92% in patency rate. We obtained the 90% patency rate results in 20 femoral artery of rats using Nakayama's

modified end-in-end technique as compared with patency rate of 85% following end-to-end techniques.

Therefore experienced advantages are:

(1) High patency rate

(2) Short operating time(20 Minutes>E-T-E technique)

(3) The possibility of clinical use in the vessels with remarkably different diameter.

## THROMBUS FORMATION

A microvascular anastomosis is a traumatic insult to a blood vessel and initiates a hemostatic response.

The surgical trauma brings the thrombus formation.

The success of microvascular surgery depends on the avoiding thrombus formation.

The early thrombus formation frequently occurs over 10 minutes at the operation and then regresses. It discovers with an intraoperative patency test (milking test) but subsequent late thrombus formation occurring in a few days has more important value in the patency.[1,9].

Although the mechanism of late thrombus formation is not known, its occurrence is probably related to the altered hemodynamics of blood flowing.

Because the mural thrombus occasionally can remains 8 to 12 days, consistent monitoring is necessary for 2 weeks.

Numerous factors can be responsible for the formation of a vascular thrombus. Acland and Trachtenberg[3](1977) concluded that thrombus was inevitable in a suture anastomosis and that it was probably initiated by the exposure of the underlying tissue collagen in the blood stream.

Van Beck ans Suchy[23], Spaet[24](1981) additionally discussed the underlying connective tissue of the vessel with its exposed collagen is changed into a thrombogenic surface which stimulates the platelets from sheroids to sticky masses with pseudopods and the platelets release sticky substances(adenosine diphosphate, serotonin).

Born and Cross[25](1964) advocated the platelet aggregates formed the blood clot, followed by organization.

Our studies has shown the following results:

Even a early thrombus formation case for 20 minutes was not found on both anastomotic techniques.

But the study of patency rate according to anastomotic methods till 3 weeks has shown differences in late thrombus formation.

The End-In-End technique has higher value of 90% (18 of 20) as compared with that of End-To-End method of 85% (17 of 20).

## WOUND HEALING OF ENDOTHELIUM

Generally arteries are classified with its size and histologic features:

(1) Large elastic arteries

(2) medium sized distributing muscular arteries

(3) arterioles

Simplistically described, blood vessels are composed of three layers(intima, media and adventitia), with each layer having a different structure and function.

The intima is a thin sheet of endothelial cell resting on internal elastic lamina.

The media is the thickest layer and is composed of smooth-muscle cells, collagen, elastic fibers and mucopolysaccharide substances.

The adventitia is composed of fiber elastic connective tissue through which nerves, lymphatic vessels pass to terminate in the media[1].

The microvascular anastomosis is surgical trauma which can injure the vascular endothelium.

The degree of endothelial cell injuries rely on the mechanical trauma, oxygen deficiency of the lumen, toxic injury of wound irrigants, invasion of leukocyte and vessel dryness.

The causes of mechanical trauma are divided into mechanical dilatation by microforceps and excessive vascular compression by clamp[26,27].

Thurston et al[4](1976) noticed of the flattening of

longitudinal ridges & sloughing in the endothelium, early thrombus occurring over twenty minutes and initiating of regeneration of the neo - endothelium for 3 days.

They also recommended the ideal vascular clamp pressure as less than 30gm/mm$^2$ in small vessels.

We applied the Acland approximator with 25gm/mm$^2$ closing pressure and single jaw clamp to reduce vessel damage.

Baechler[28](1977) reported that the temporary oxygen deficiency in the anastomotic site causes the sloughing of endothelium.

Vascular surgeons have studied the value of topical irrigant, thrombolytic agents[3,28].

Stewart et al[5,29](1977) studied the vascular endothelial damage produced by migration and sticking of leukocytes occurring in suture site.

O'Brien[11](1970), Minder jahr[30](1979), Jacobon and Suarez[31] have noted that the wall of microvessels consist of collagen fibers, basement membrane, elastic fibers, non - thrombogenic endothelial cell and the wall can be converted into thrombogenic surface by exposure to the blood stream.

In our studies, the injury of endothelium could be decreased by use of heparinized saline(1,000U/100mℓ). Shorting of operating time by E - I - E technique could be advantageously in decreasing low oxygen condition in the vessel.

Numerous studies on the histology of endothelial healing process have been reported.

Walsh and Barnhart[32] noted the thrombus covering the anastomotic vessel originated from the platelet aggregates and the thrombus growth continued till 8 - 12 days.

Lauritzen and Johansson[33](1980), Lauritzen and Bagge[34](1979) had awared of the healing results being achieved with polymorpholeukocyte, moncoyte about one week on the vascular suture site.

Wojik et al[35] had reported the role of platelet which had pseudopods, covered the injuried endothelium and changed the wall as non - thrombogenic pseudo - intima.

It has been variously proposed that new endothelial cell arise from adjacent endothelial cells or from circulating mononuclear cells, or from smooth muscle cells of media[4,33,36,37,38,39].

The report that new endothelial cells are similiar to the fibrocyte also presented by Acland and Trachtenberg[3](1977).

Reendothelialization period of the lumen remains as debating problems. Repair of the intima & media advocated by Thurston et al[4](1976) initiated during first to second week.

Nightingale and Fogdestam[5](1980). Harashina and servant[26](1976) have observed that during the fourth week, the media was restored by a smooth muscle proliferation.

Milward[38](1976) reviewed histologically that endothelial regeneration in small vessels have undergone completely in sixth week.

Our studies have shown that the repair of damaged intima occurred for 1 - 2 week in E - I - E group, whereas for 2 - 3 week in E - T - E group and the fact that monocyte - like celll immerged into the vessel wall in the E - I - E group found on postoperation 3 weeks was in concord with the Milward and Rayner's views[38](1976).

The hypothesis that the monocyte may be the origin cell of neo - endothelium could be achnowledged. But this experiment was not ascertained to the theory.

## ATROPHY OF MEDIAL LAYER OF ENDOTHELIUM

Minderjahr and Dahm[30](1979) also reviewed histopathologically 50 cervical arteries of rats.

The examination disclosed the extended area of atrophic medial layer at the site of injury.

Khodadad[40](1970) evaluated microvascular repair replacement after end - to - end anastomosis in the long term study. Replacement phenomenon of atrophic medial layer into fibrous tissue was observed

at 11-23 months in the suture circle.

Our study accidently showed the disrupition of medial layer in the E-I-E group on the postoperation 1 week.

In the analysis, the etiology of atrophy of medial layer was due to partial interruption of blood flow to the stripped adventitia wall, accidental damage of vessel handiing. But the medial necrosis was considered as inevitable role in the widening of lumen in the E-I-E group.

The results of our experimental study can be insufficient histological examination, but the results suggested the possibility that E-I-E technique also can be the available microvascular technique to the large blood vessel size discrepancy, difficult situation in access to the suture site as E-T-E technique, vein graft operation case.

## V. CONCLUSION

The microvascular anastomoses have been performed with End-In-End method in the femoral arteries of 20 rats group, also with End-To-End on the same arteries of another 20 rats group, and then the four anastomosed vessels in 5 subdivided group on each group were taken after period of 1, 3, 7, 14, 21 days following by anastomoses for scanning electron microscopic observation.

the results were as follows:

1. The patency rate was 90% in the EIE group and 85% in the ETE group and late thrombus occurred at 7, 14 days on both groups, which suggested consistent monitoring of patency was required for two weeks at least.
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3. Re-endothelialization was completed between 7 and 14 days in EIE group, whereas between 14 and 21 days in EIE group.

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국문초록

# 백서 대퇴동맥에서의 혈관함입문합술과 혈관단단문합술의 주사전자현미경적 비교연구

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미세혈관봉합술에서의 가장 큰 문제점은 봉합부에서의 내피손상과 혈전형성이라고 볼 수 있다. 이 연구의 목적은 봉합시 일어날 수 있는 내피손상부에서의 치유과정을 관찰코져 각각 다른 문합술인 혈관함입문합술과 혈관단단문합술을 백서 대퇴부동맥에 적용하여 개존율및 전자현미경적 관찰을 통하여 비교하였고 아울러 임상에의 적용 가능성을 검토코져 하였다.

저자는 미세현미경시야에서 혈관함입문합술 20례와 단단문합술 20례를 시행한후 1일, 3일, 1주, 2주, 3주에 각각 4마리씩 희생후 문합혈관부를 육안관찰후 주사전자현미경으로 조직변화를 관찰하여 다음의 결과를 얻었다.

1. 혈관 함입문합술 시술시 문합후 개존율은 90%였고 혈관 단단문합술은 85%였다.
2. 혈관 함입문합시 술후 3일째는 문합부에서의 혈소판 응집물이 기질화되었으며 함입으로 좁아져 있던 혈관내경이 약 1주째 혈관 함입부의 중막 위축현상으로 다소 넓어졌다.
3. 혈관 내피재생과정은 혈관 함입문합술에서는 7일에서 14일경에, 혈관 단단문합술에서는 14일에서 21일째 완성되었다.

## Explanation of Photomicrographs

Photo. 1. Arterial endothelium of the rat. Smooth luminal surface with longitudinal ridges(arrows) is seen(X 500).

Photo. 2. Higher magnification view of endothelium. The surface of endothelial cells is covered with microvilli(open arrow). Endothelial cell(EC) is rhomboid in shape. Mechanical injury by forceps also can be seen(arrow)(X 1500).

Photo. 3. 1 day after EIE anastomosis. Inserted proximal vessel end lying within the distal lumen is seen(open arrows). (X 500).

Photo. 4. Highter magnification view(1 day). Platelets and red cells on the injured lumen area(arrow)(X 2000).

Photo. 5. 3 days after EIE anastomosis. Macrophages, monocytes(uppermost arrow) can be seen on the distal end of inserted vessel. Middle, lowermost arrows indicate the border of vessel end(X 1400).

Photo. 6. Higher magnification view of the lumen(3 days). Conglomeration of macrophage(M), desquamated endothelial cell(open arrow), monocytes in the lumen(X 2100).

Photo. 7. 1 week after EIE anastomosis. Atrophy of medial layers of the wall is seen(open arrow). Re-endothelialization partially occurred(arrow)(X 700).

Photo. 8. Higher magnification view of the lumen(1 week) Flat shaped-neointimal cells(NC) spreaded on the end of vessels(X 2500).

Photo. 9. 2 weeks after EIE anastomosis. The luminal surface shows complete reendothelialized state of endothelium. The diameter of the anastomosis site(arrows) is wider than that of vessel lumens(open arrows). The fibrosis of outer wall(X) is also seen(X 500).

Photo. 10. Higher magnification view of the end of inserted vessel(2 weeks) Medial layer(O), hyperplasia of neointimal layer(X) indicated(X 2500).

Photo. 11. 3 weeks after EIE anastomosis. The thinning of the inserted vessel wall(X) is seen(X 500).

Photo. 12. Higher magnification view of the lumen(3 weeks). The end of inserted vessel is continuous smoothly with distal vessel wall. The depicted site as "X" is the border of inserted vessel end(X 2000).

Photo. 13. 1 day after ETE anastomosis. Mural thrombus(arrow) consisted of platelets, RBC. The distortion of vessel wall due to tight suturing also advocated(X 2200).

Photo. 14. 3 days after ETE anastomosis. The thrombus still remains on the thread, needle punctured areas(arrow). Desquamated endothelial area also is seen(open arrow)(X 1500).

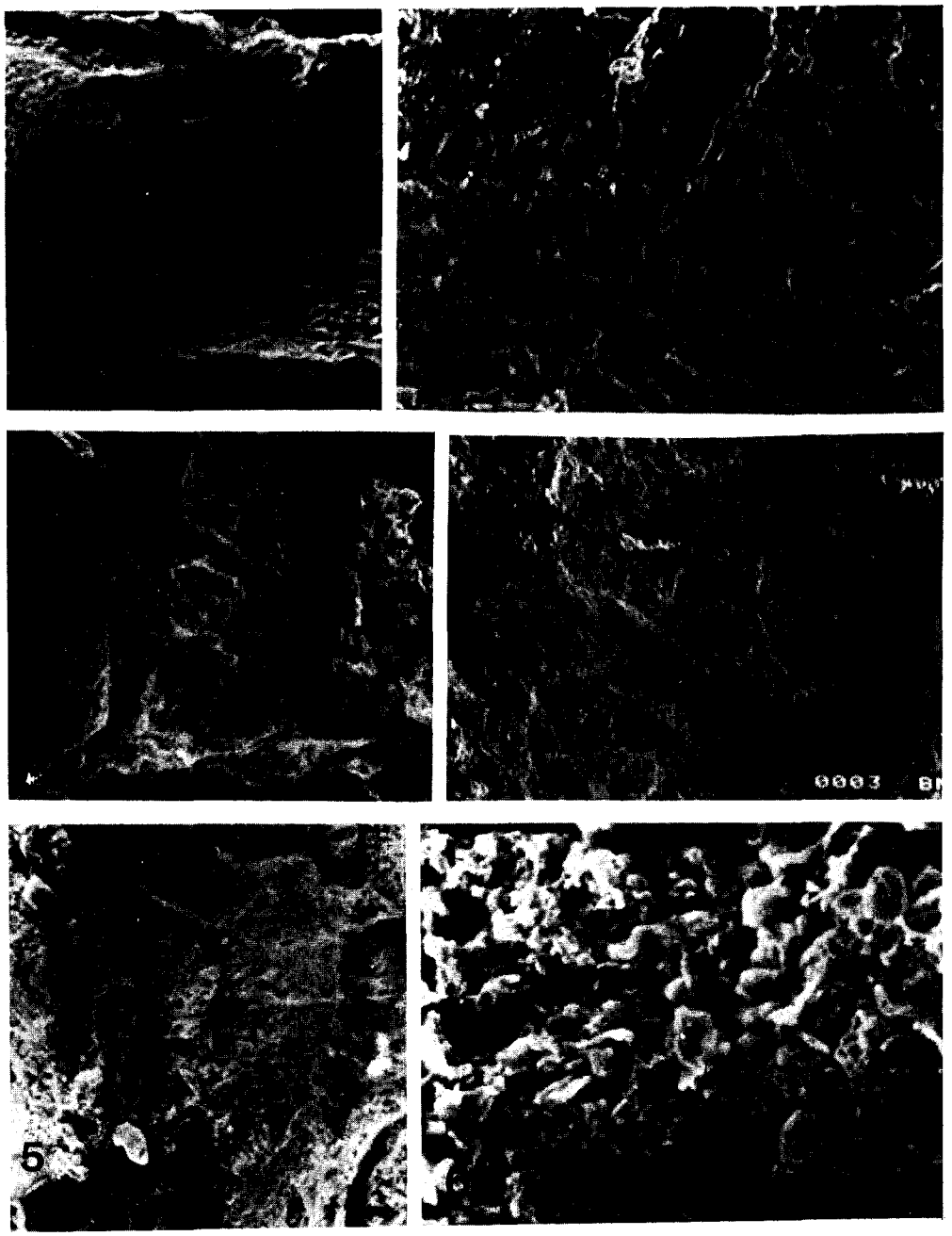
Photo. 15. 1 week after ETE anastomosis. The thrombus disappeared, and re-endothelialization is beginning partially(X 1300).

Photo. 16. 2 weeks after ETE anastomosis The thread is covering with neointimal layer. Reendothelized area(arrow) is seen(X 1400).

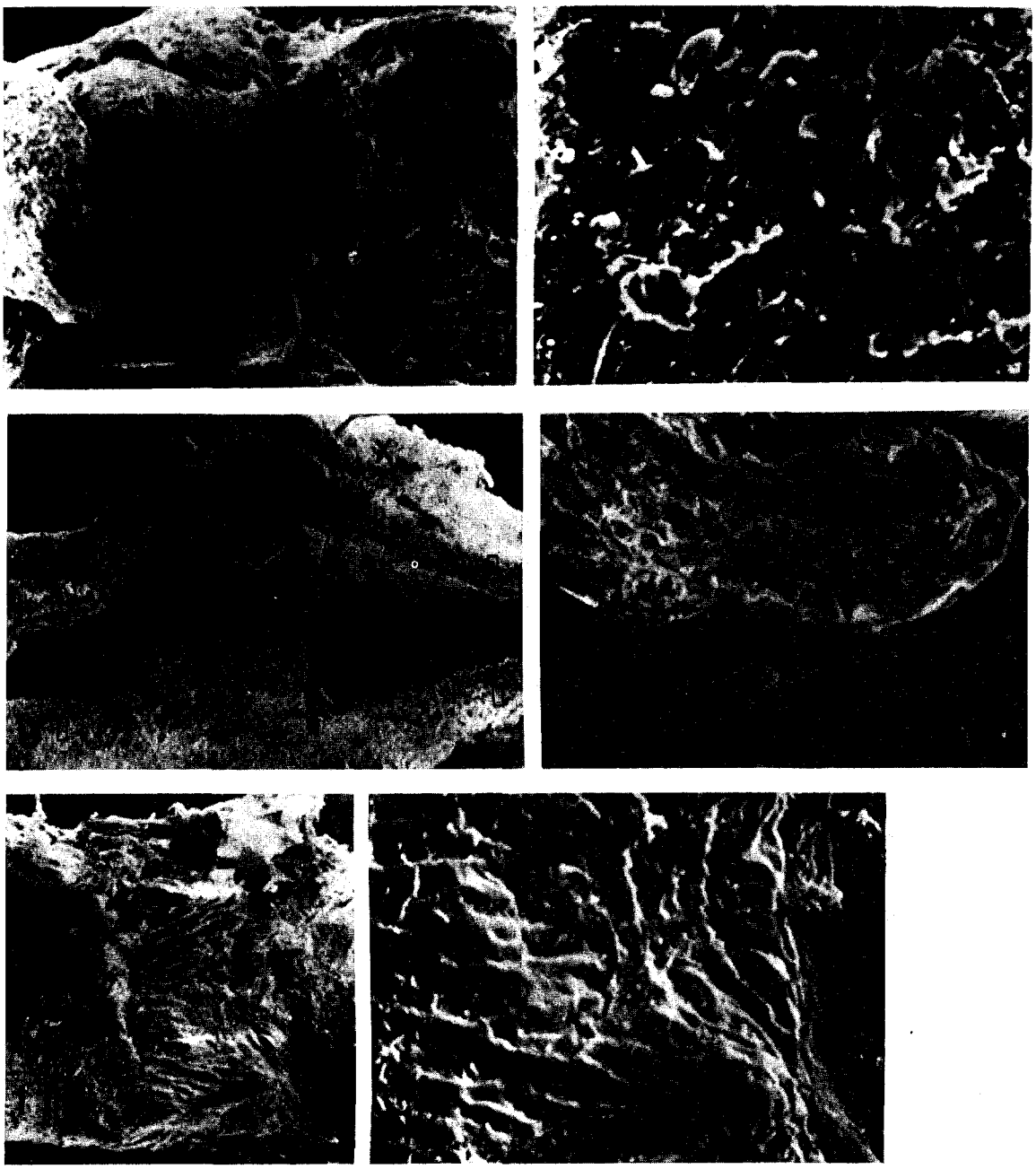
Photo. 17. 3 weeks after ETE anastomosis. Complete healing of endothelium is certified by smooth luminal surface, longitudinal ridges. Monoocyte-like cells submerged on the vessel wall (arrows))X 1300).

Photo. 18. Higher magnification view of the lumen(3 weeks). Monocyte-like cell(arrow) is seen(X 3000).

Photomicrograph ①



Photomicrograph ②



Photomicrograph ③