doi: 10.30934/kusbed.691901 **e-ISSN:** 2149-8571

Kocaeli Üniversitesi Sağlık Bilimleri Dergisi

Özgün Araştırma / Original Article

http://dergipark.gov.tr/kusbed

A NOVEL END-TO-END ANASTOMOSIS TECHNIQUE IN VESSELS SMALLER THAN ONE MM DIAMETER: THE ZIGANA METHOD

ÇAPI BİR MM'DEN DAHA KÜÇÜK DAMARLARDA YENİ BİR UÇ UCA ANASTOMOZ TEKNİĞİ: ZİGANA TEKNİĞİ

Emrah Kağan Yaşar[1*], Murat Şahin Alagöz[1],

[1]Kocaeli University, Department of Plastic Reconstructive and Aesthetic Surgery, Kocaeli, Turkey

ORCID iD: Emrah Kağan Yaşar: 0000-0003-0572-1248; Murat Şahin Alagöz: 0000-0002-5521-7173

***Sorumlu Yazar / Corresponding Author:** Emrah Kağan Yaşar, **e-posta / e-mail:** emrahkaganyasar@hotmail.com

Geliş Tarihi / Received: 23.05.2020 **Kabul Tarihi / Accepted:** 24.11.2020 **Yayım Tarihi / Published:** 03.01.2021

Abstract

Introduction: Traditional end-to-end microvascular anastomoses, the sine qua non of reconstructive microsurgery, has disadvantages, such as narrowing in the anastomosis line, excessive suture use, and risks of rotation and injuring the posterior wall especially for vessels with small diameter. It is aimed to compare the traditional method with a novel end-to-end vascular anastomosis technique suitable for use in very narrow vessels.
Methods: Fifteen female Wister Albino rats weighing 235-275 g were used. The experimental (Zigana) technique was applied to the right femoral artery, and the traditional anastomosis technique to the left. Operative time, suture counts, permeability rates and bleeding time were analyzed at clinical evaluation, and endothelialization, inflammation, fibrosis, foreign body reaction, intimal hyperplasia, intimal injury, thrombus and necrosis at histopathological evaluation.
Results: Clinical evaluation revealed a longer operative time, lower suture count, and a shorter bleeding time with the Zigana technique, while permeability rates were the same with both methods. Similar thrombus, stenosis, inflammation, fibrosis, and foreign body reaction rates were observed in both groups at histopathological examination. Intimal hyperplasia rates were lower in the Zigana technique group, while no necrosis was encountered in either.
Conclusion: The Zigana Technique exhibited better suture use and bleeding time values than the traditional method, although the difference between the two groups was not statistically significant. It is thought that the Zigana technique, which can yield positive results even with vessels less than 1 mm in diameter, is capable of use in microvascular anastomoses in both clinical and experimental studies.

Keywords: *End-to-end anastomoses, microvascular surgery, reconstructive microsurgery*

Öz

Amaç: Rekonstrüktif mikrocerrahinin olmazsa olmazı olan, geleneksel uç uca mikrovasküler anastomozların anastomoz hattında daralma, aşırı suture kullanımı ve damarda rotasyon ve özellikle küçük çaplı damarlarda arka duvar hasarı gibi dezavantajları mevcuttur. Bu sebeple küçük çaplı damarlarda kullanılabilecek yeni bir yöntemin geleneksel uç uca anastomoz tekniğiyle karşılaştırılması amaçlandı.
Yöntem: Bu çalışmada 15 Wistar Albino cinsi sıçan (235-275g) kullanıldı. Sağ femoral artere deneysel yöntem (Zigana), sol tarafa ise geleneksel yöntem uygulandı. Klinik değerlendirmede ameliyat süresi, dikiş sayıları, geçirgenlik oranları ve kanma zamanı; histopatolojik değerlendirmede endotelizasyon, inflamasyon, fibrosis, yabancı cisim reaksiyonu, intimal hiperplazi, intimal yaralanma, trombüs ve nekroz varlığı analiz edildi.
Bulgular: Klinik değerlendirmede, Zigana yöntemi ile daha uzun ameliyat süresi, daha düşük dikiş sayısı ve daha kısa kanama süresi ortaya çıkarken, geçirgenlik oranları her iki yöntemde de aynıydı. Histopatolojik incelemede her iki grupta da benzer trombüs, stenoz, inflamasyon, fibrosis ve yabancı cisim reaksiyon oranları gözlendi. Zigana yöntemi grubunda intimal hiperplazi oranları daha düşükken, hiçbir sıçanda nekroz izlenmedi.
Sonuç: Zigana yöntem`i, geleneksel yönteme göre daha az dikiş kullanımı ve daha kısa kanama zamanı değerleri sergilemiştir, ancak iki grup arasında istatistiksel anlamlı fark bulunmamaktadır. Çapı 1 mm`den daha küçük damarlarda bile olumlu sonuç verebilen Zigana yöntemi`nin hem klinik hem deneysel çalışmalarda kullanılabileceği düşünülmüştür.

Anahtar Kelimeler: *Uç uca anastomoz, mikrovasküler cerrahi, rekonstrüktif mikrocerrahi*

Introduction

Microvascular anastomoses was first made possible with the surgical use of microscopes, and end-to-end anastomoses of 1.4-mm diameter vessels were successfully carried out in 1973.[1,2] Studies showed that 15% narrowing may occur in the vessel lumen with traditional end-to-end anastomosis, the most frequently applied method.[3,4] Additionally, in the traditional method, the risk of posterior wall injury increases as the vessel diameter decreases. In case of a long pedicle in free flap surgery, rotation and strangulation may occur due to orientation difficulties in the vessel ends. The purpose of this study was to compare the traditional method with a novel anastomosis technique in vessels with a diameter of 1 mm or less in which such potential risks that may be encountered in traditional end-to-end anastomosis may be greater than normal.

Methods

This experimental study was performed at the Kocaeli University Experimental Medical Research and Application Unit (DETAB) Turkey, following receipt of approval from the Kocaeli University Animal Experiments Local Ethical Committee (No. 10/2).

Fifteen female Wistar Albino rats weighing 235-275 g were used. Experimental anastomosis was applied to the right femoral arteries and traditional anastomosis to the left femoral arteries of each animal. Following preparation of the femoral arteries, the diameters were measured and recorded for each rat, and ranged between 0.4 and 1 mm.

Surgical Techniques

Following dissection of the femoral region, the vessel ends were released for the traditional method, vasodilation was performed with dilator forceps, and intraluminal washing was performed with heparinized saline solution and 10% lidocaine. The adventitia was cleared up to the diameter of the vessel ends. Anastomosis was completed using eight sutures with the 0-180 suture technique and 10-0 polyamide microsutures. Vessels undergoing this procedure constituted the control sample group.

For the experimental procedure, following similar preparations to those in the traditional method, bilateral fish mouth incisions were made at a distance up to the radius of the vessel lumen, and four vessel flaps were obtained. Diagonal flaps were removed and prepared for anastomosis. The technique described is shown in schematic form in Figure 1.

Anastomosis was completed using eight sutures, first to the flap corners, and then to the intermediate spaces. Once the approximator clamp was released, the fat pad was placed on the anastomosis line. The repair was observed for the first 3 minutes post completion, and if leakage occurred, this was halted with additional sutures. The anastomosis line was rechecked at 3, 5 and 15 min, and continuity of flow was confirmed using the milking test. A chart of the experimental method stages and applications is shown in Figure 2.

Evaluations

Anastomoses were evaluated in terms of four clinical parameters. Time from attachment of the approximator clamp to the femoral artery to completion of anastomosis and release of the approximator was defined as Operative Time, and the number of suture knots applied at each anastomosis was recorded as the Suture Count. Our target was to apply a total of eight sutures in both groups. Extra sutures applied in the event of uncontrollable leak were subsequently added to the count. Third, patency was checked at 3, 5, and 15 min and on day 15 using the milking test. The results were recorded as present or absent under the term Patency Rate. Duration of blood leakage from the anastomosis line after completion of anastomosis was recorded. Following completion of anastomosis, leakage from the anastomosis line was checked by lifting the fat pad at 30-sec intervals. Time when leakage stopped was recorded as Bleeding Time.

One-centimeter vessel biopsies were collected from all rats on day 15 postoperatively for histopathological evaluation, after which the rats were sacrificed. Sixteen randomly selected specimens, eight from each group, were evaluated under a light microscope. Specimens with macroscopic aneurysm and anastomoses with negative patency, the numbers of which were equal between the groups, were not included in the histopathological examination. Specimens were evaluated under a light microscope and compared in terms of endothelialization, fibrosis, foreign body reaction, necrosis, intimal injury, intimal thickening, and lumen stenosis.

Serial sections, 3 micrometers in thickness, were collected from the specimens for histopathological examination. The anastomosis line was identified, and sections were stained with hematoxylin eosin (HE) and silver staining (SS). The prepared sections were examined under an Olympus BX53 model microscope receiving daylight (Olympus Corporation, Shinjuku Monolith, 3-1, Nishi Shinjuku 2-chome, Shinjuku-ku, Tokyo, Japan).

Results

Clinical Findings

Data were compared using chi-square statistical analysis, and no statistically significant differences were determined in terms of operative times, suture counts, bleeding times, or permeability ($p<0.001$ for all).

One anastomosis with negative patency was present in each group. Patency was positive in all but two rats, one from each group, at milking tests performed on day 15. Flow permeability was observed in all other rats. Clinical findings for the two groups are shown in Table 1.

Four macroscopic aneurysms with positive patency, two from each group, were observed. These specimens are shown in Figure 3.

Light Microscopy Findings

In the control group, a clear lumen and adequate endothelialization were generally observed, and specimens with minimal intimal hyperplasia predominated. Intimal thickening and luminal narrowing were observed in addition to acute inflammation in this group. Thrombus almost totally obstructing the lumen and areas of neovascularization inside this thrombus were observed in one sample, accompanied by a loss of endothelial integrity in the same area. Luminal narrowing was observed in three specimens, two mild and one very severe. Luminal narrowing was accompanied by absence of endothelialization and minimal intimal thickening.

In the experimental group, specimens with endothelialization and a clear lumen again predominated. While intimal hyperplasia was present, this was less severe

than in the control group. Advanced luminal narrowing, impaired endothelial integrity, and heterogeneous intimal hyperplasia were observed in one sample. Chronic inflammation was also observed in one specimen. Histopathological examination specimens from both groups are shown in Figure 4.

Table 1. Comparison of operative times, suture numbers, bleeding times, and permeability rates. When the values were compared statistically, there was no significant difference between the two groups

	Group 1 - Experimental (Mean ± SD)	Group 2 – Control (Mean ± SD)
Operative time (min)	36.33 ± 3.01	34.06 ± 3.32
Suture count (n)	8.46 ± 0.83	8.86 ± 0.91
Bleeding time (min)	1.86 ± 0.58	2.36 ± 1.36
Permeability rate (%) (3 min- 5 min-15 min, and 15 days)	93.3- 93.3- 93.3. 93.3	93.3- 93.3- 93.3- 93.3

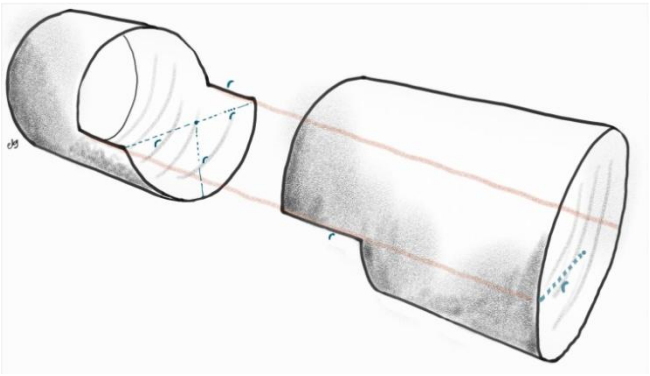

Figure 1. Schematic representation of the experimental Zigana method. Fish mouth incisions were made at a distance equal to the radius of the vessel lumen and a total of four vessel flaps were obtained. Before beginning microvascular anastomosis, cross-selected flaps are removed horizontally.

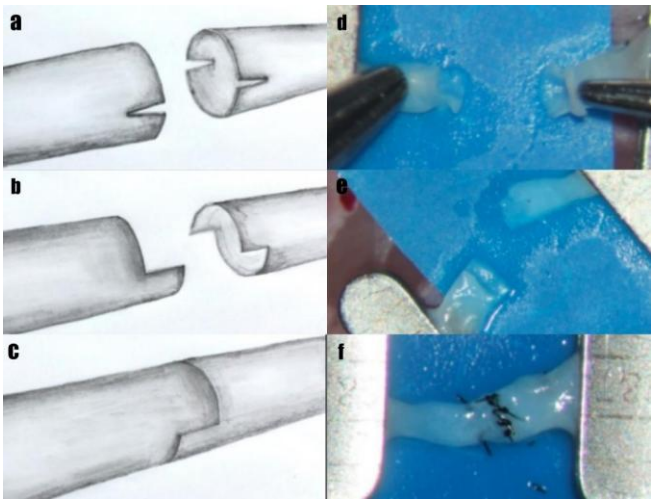

Figure 2. Pre-anastomosis preparations and post-anastomosis appearance in the experimental method. A,B,C are schemas and D,E,F are peroperative photographs of experimental method stages.

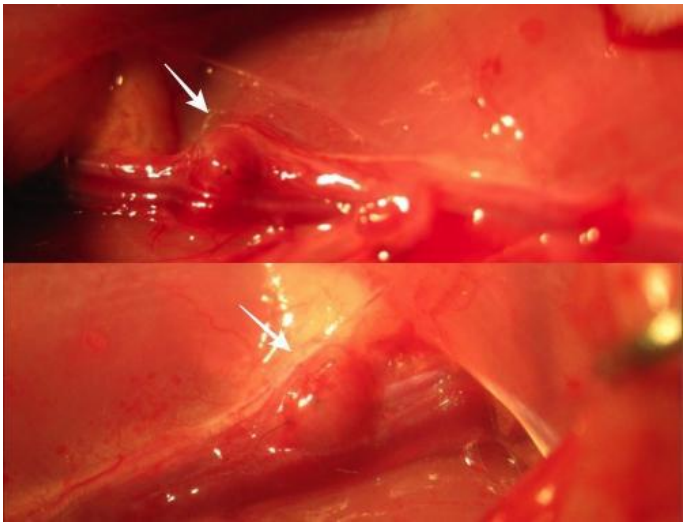

Figure 3. Macroscopic aneurysms observed on day 15 in the experimental group (top) and the control group (bottom)

Discussion

An atraumatic procedure is the most important condition for success of anastomosis in microvascular surgery.[5-7] The probability of surgical success increases in line with the vessel diameter. However, as the vessel diameter decreases, the difficulty of the procedure rises, and the risks of intravascular thrombus development and vascular stenosis increase. In the traditional end-to-end anastomosis method, the most popular technique in microvascular surgery, in addition to 15% lumen narrowing, the probability of thrombus formation in the lumen with the assistance of vasospasm is higher in small diameter vessels than in large vessels. Another known disadvantage of the traditional end-to-end anastomosis technique is that the possibility of passing through the posterior wall increases as the vessel diameter decreases. This not only increases the risk of thrombus formation, but may also necessitate repeat sutures for successful anastomosis. In this experimental method involving small vessels less than 1 mm in diameter, since the surface of the anastomosis was not in a single plane perpendicular to the longitudinal line of the vessel, narrowing in the anastomosis line may have been less than in the traditional method. Moreover, the fact that the vessel ends are not directly contiguous with the posterior walls may provide easier posterior wall control.

For an anastomosis to be regarded as ideal, the anastomosis time must be short, the number of sutures passing through the intralumen must be low, less manipulation of the vessel ends is required, fewer sutures must be used, intima and media layer separation rates must be low, and diameter mismatch must be successfully resolved.[8] In the experimental method, the flaps in the vessel end must be obtained atraumatically using sharp micro scissors. The smooth muscle connections and severity of circular muscle spasms that may occur in the anastomosis line can be reduced with fish mouth incisions up to the radius in the vessel ends. The experimental method was not statistically significantly different in terms of the suture material used.

However, the suture count was higher due to the difference in the additional suture material. Due to the preparation of the vessel ends, the application of the experimental procedure lasted longer than the traditional method, and although the difference was not statistically significant, the longer procedure time is a disadvantage of the experimental method.

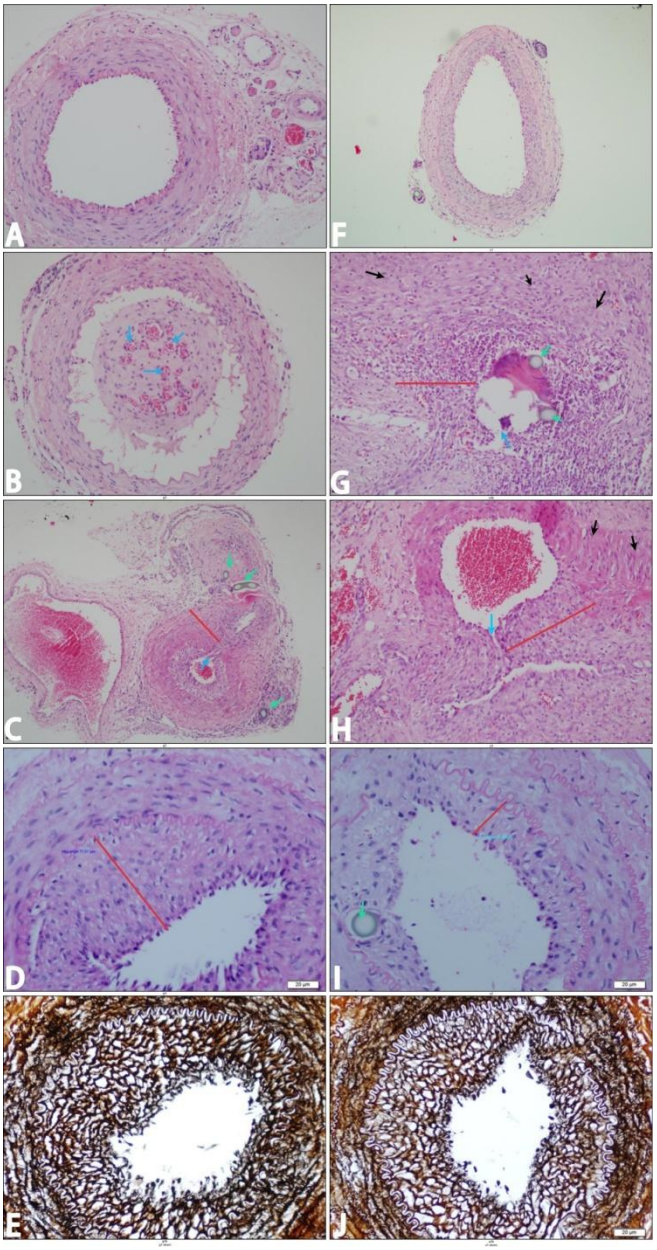

Figure 4. Histopathological specimens (All specimen but E and J are Hematoxylin eosin (HE) stained specimens. Figures E and J are Silver reticular staining (SRS) specimens).
Control group (traditional end-to-end anastomosis) (left)
A. Clear lumen with normal histological findings (x200 magnification)
B. A case ending in post-thrombus recanalization (blue arrow), showing impaired endothelial integrity (x200 magnification)
C. Luminal narrowing (blue arrow), intimal hyperplasia (red line), and suture materials exhibiting surrounding foreign body reaction (green arrows) (x200 magnification)
D. 77-micrometer thick intimal hyperplasia (red line) (x400 magnification)
E. 77-micrometer-thick intimal hyperplasia (x400 magnification)
Experimental group (Zigana Method) histopathological images (right)
F. Intimal thickness within normal limits and suture material (x100 magnification)
G. Luminal narrowing (blue arrow), intimal hyperplasia (red line), and chronic inflammation (black arrows) (x200 magnification)
H. Luminal narrowing (blue arrow), intense inflammation (large black arrows) and sutures (green arrows) with surrounding foreign body reaction (x200 magnification)
I. 36-micrometer intima layer (red line) and suture material (green arrow) (x400 magnification)
J. Intima layer showing minimal hyperplasia (x400 magnification)

The sutures placed in the anastomosis line must be full thickness, and the distance from the suture to the vessel must be twice the arterial thickness in the artery or the venous thickness in the vein.[9] If this distance is less than required, tears may develop in the ends, while if it is greater than necessary, leaks in the anastomosis line may decrease, but the vessel ends may undergo inversion.[10] Intraluminal material must be used as little as possible in order to establish rapid endothelialization, to reduce the risk of thrombus, and to reduce the risks of late stage inflammation and giant cell formation to a minimum. Although the difference was not statistically significant, less intraluminal material was used in the experimental method in this study, due to the low suture count. No significant difference was also observed between the two groups in terms of luminal stenosis, thrombus, inflammation, or foreign body reactions. Similar results to the traditional method were achieved in terms of endothelialization. In the experimental method, we believe that following a minimal learning curve and acquisition of a suitable level of experience with this technique, with the straight incisions successfully applied during the preparation of the vessel ends, the torsion and strangulation risks that may frequently be encountered in the traditional method can be reduced.

In the traditional method, no loss in vessel length occurs during the preparation of the vessel ends. In the experimental method, however, a distance equivalent to the radius (r) is lost in the vessel ends. If the anastomosis fails, since renewing the vessel ends and once more preparing the ends using the same technique for anastomosis revision will result in loss of distance equivalent to the radius on every occasion, problems will emerge with obtaining a sufficiently long and non-tense anastomosis. Atraumatic activity in the preparation of the vessel end flaps is of great important for prevention. The probability of anastomosis success will be high with the preparation of regular flaps exhibiting complete orientation to one another, such as to prevent rotation and sutures passing through the posterior wall. However, the novel technique described is not recommended for surgeons with insufficient microsurgery experience newly embarking on such procedures. Nevertheless, we recommend that it be borne in mind in microvascular anastomoses smaller than 1 mm in diameter.

Comparing the anastomosis surface in the two techniques, an extra length up to the radius (2r) is present in the anastomosis in the new technique. That length may increase the relative possibility of thrombus formation and may be regarded as a disadvantage of the technique. However, we observed no statistically significant difference between the two groups in terms of flow continuity and thrombus formation.

The technique described here was applied to the arteries and compared with the traditional technique. Vein walls are thinner than arterial walls, and venous flow velocity is slower. Vein walls being thinner and more fragile may result in technical difficulties for the surgeon in the preparation of vessel and flaps and during adventiectomy, as well as prolonging the duration of anastomosis. In addition, the anastomosis surface, being one radius greater than in the traditional method and the intravascular flow velocity being lower, can increase the possibility of thrombus formation. However, we think that there is no theoretical obstacle to the technique being applied to veins. Further studies evaluating the experimental technique in terms of vein anastomoses are suggested.

In anastomoses with equal vessel diameters, vessel end flaps of equal length occur with full fish mouth incisions. Despite technical difficulties, the technique can be applied in vessels with a low difference in diameters. We think that the difference in diameter between length of the flap as far as r^1 in large diameter vessels and the length of the flap to be applied close to r^1 in small diameter vessels can be reduced. However, we do not think that the experimental method is practical in overcoming a large diameter difference.

Conclusion

The 'Zigana' method described in this paper is a technique that can be applied by surgeons who have completed basic microsurgery training and with some experience of microsurgery. Disadvantages such as stenosis in the anastomosis line, difficulty in protecting the posterior wall, the risk of torsion, and anastomosis with high suture numbers can be avoided with the Zigana method. Mutual opposition of the vessel ends in a lock and key form prevents these disadvantages, and posterior wall protection is high since two dimensions in the anastomosis line are eliminated. The integrity of circular smooth muscles is eliminated by means of the incisions, and narrowing in the anastomosis line can be prevented.

Our evaluations revealed no statistically significant difference between the Zigana and traditional methods. Despite being very close to one another, the extra suture requirement was lower, and similar results were obtained in terms of thrombus formation. The Zigana method entails such disadvantages as the vessel preparation time and total surgery time. Once sufficient experience has been acquired, progress can be made in terms of such disadvantages such as preparing the vessel ends and total anastomosis time. Clinical, statistical, and microscopic analyses suggest that the Zigana method is an easily applied anastomosis technique for microsurgeons with moderate to high levels of experience that reduces foreign body material, exhibits high permeability rates, and is capable of reducing the risks of posterior wall damage and rotation.

Conflict of Interest

The authors have no conflicts of interest to disclose.

Author Contributions

EKY, MŞA: Design; EKY, MŞA: Project development; EKY: Literature search; EKY: Analysis; EKY: Manuscript writing; EKY, MŞA: Critical review

References

1. Buncke HJ Jr, Schulz WP. Total ear reimplantation in the rabbit utilising microminiature vascular anastomoses. *Br J Plast Surg*. 1966 Jan;19(1):15-22. doi: 10.1016/s0007-1226(66)80003-6. PMID: 5909469.
2. Rickard RF, Hudson DA. A history of vascular and microvascular surgery. *Ann Plast Surg*. 2014 Oct;73(4):465-472. doi:10.1097/SAP.0b013e31827 10027. PMID: 23318371.
3. Bschorer R, Frerich B, Wolburg H, Gehrke G, Schwenzer N. Fibrin sealing and histometrical changes in conventionally sutured microvascular anastomoses. *J Craniomaxillofac Surg*. 1993 Jul;21(5):192-198. doi: 10.1016/s1010-5182(05)80480-9. PMID: 8360351.
4. Siemionow M. Histopathology of microarterial anastomoses: end-to-end versus end-in-end (sleeve) technique. *J Hand Surg Am*. 1990 Jul;15(4):619-625. doi: 10.1016/s0363-5023(09)90025-9. PMID: 2380526.
5. Cobbett J. Small vessel anastomosis. A comparison of suture techniques. *Br J Plast Surg*. 1967 Jan;20(1):16-20. doi: 10.1016/s0007-1226(67)80003-1. PMID: 5339722.
6. Khouri RK. Avoiding free flap failure. *Clin Plast Surg*. 1992 Oct;19(4):773-81. PMID: 1339635.
7. Eisenhardt HJ, Hennecken H, Klein PJ, Pichlmaier H. Experiences with different techniques of microvascular anastomosis. *J Microsurg*. 1980 Mar-Apr;1(5):341-350. doi: 10.1002/micr.1920010503. PMID: 7441162.
8. O'Brien, B.M., Morrison, W.A., Reconstructive Surgery. Churchill-Livingstone. *Edinburgh*, Page 3-113,179-181,225, 1987
9. Demiralp CO, Serel S, Zeyrek T, Demirseren ME, Ergun H, Yormuk E. New end-to-end microvascular anastomosis with geometrically adaptable ends technique: an experimental study on rats. *Bratisl Lek Listy*. 2011;112(9):483-487. PMID: 21954523.
10. Harashina T, Fujino T, Watanabe S. The intimal healing of microvascular anastomoses. *Plast Reconstr Surg*. 1976 Nov;58(5):608-613. doi: 10.1097/00006534-197611000-00012. PMID: 790429.