Comparison of the Microvascular Anastomosis Maturation in Continuous and Interrupted Suture Technique.

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Research Article

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Abstract

BACKGROUND

Use of interrupted sutures in microvascular anastomosis is widely accepted standard technique. Usage of continuous running suture (CRS) is less common due to the presumption that its mechanical firmness can negatively affect anastomosis maturation, but shorter surgical time can be an important factor in the prevention of tissue ischemia. Purpose of this study was to determine whether the use of CRS allows microvascular anastomosis maturation.

MATERIAL AND METHODS

A rat common carotid artery (CCA) end-to-end microanastomosis model was used, with 19 Long-Evans rats in the interrupted suture group and 13 Long-Evans rats in the CRS group. An immediate blood flow of the operated and the contralateral intact CCA was compared to each other at the time of the anastomosis completion and after 14 days. Quantitative transit time flowmetry measurement and histological examination were used.

RESULTS

Initial blood flow in both intact CCAs was equal in all animals (p =.004). In the interrupted suture group, the median anastomosis blood flow was 88.9% of the contralateral CCA, median time needed for the suture was 46 minutes. The blood flow after two weeks increased to 96.1%. In the CRS group, the median anastomosis blood flow was 88.3% of the contralateral CCA, median time needed for the suture was 30 minutes. The blood flow after two weeks increased to 100.0%. Achieved suture time reduction was 34.8% (p <.001). Histological examination showed a mature scar.

CONCLUSIONS

In our study, CRS and interrupted suture microanastomosis maturation rate was comparable. Use of CRS allowed a significant surgical time reduction.

Introduction

Precise surgical technique is the key in creating a functional microvascular anastomosis. In order to achieve maximal possible blood flow, the anastomosis site must be able to expand over time. Mechanical factors leading to anastomosis maturation are arterial wall pulsatility and direct effect of blood pressure on the site of anastomosis. Use of interrupted sutures is the widely accepted standard technique. Presumed advantages are the ability of maturation and simple repair of technical errors. Usage of a continuous running suture (CRS) is less common. Presumed advantages are time efficiency[1] and bleeding reduction at the site of the anastomosis. Shorter surgical time is an important factor in the prevention of tissue ischemia[2]. However, it is unclear whether the continuous character of a running suture might negatively affect anastomosis elasticity, thus leading to restriction of maturation of the anastomosis. In our study, maturation of the microvascular anastomosis was monitored in a rat model. An end-to-end anastomosis of the rat common carotid artery (CCA) acts as a suitable laboratory microanastomosis model[3].

In order to quantitatively assess blood flow changes in time, transit time flowmetry (TTF) measurement was used concurrently with histological examination of the arterial wall. The aim of this study was to determine whether both interrupted suture and CRS technique provide a suitable environment for microanastomosis maturation.

To authors' knowledge, no similar study using exact quantitative methods in measurement of delayed maturation of microanastomosis has been published.

Materials And Methods

Graphical representation of the methods of the study is shown as Figure 1.

Animals

Adult SPF Long-Evans rats of both sexes, weighing 400-650 g and aging 5 months (± 1 month), were used in the experiment. Common carotid artery diameter ranged between 0.7-1.2 mm and the vessel was of sufficient length, without branches.

The rats were housed individually throughout the experiment and received a standard of care according to EU directive 2010/63/EU, including a 12/12 light schedule and free access to pelleted food (Sniff, Germany) and water.

The experiment was preceded by a pilot study focused on practicing surgical techniques and optimizing the methodology.

In the main study, the animals were randomly divided into two groups based on the type of suturing technique used. Group 1, consisting of 19 animals, underwent an anastomosis using the interrupted suture technique (Table 1). Group 2, consisting of 13 animals, underwent an anastomosis using the CRS technique (Table 2). Randomization via a coin toss determined which CCA was operated and which was used as a control.

Table 1
Interrupted suture technique group

		First surgery							Second surgery		
		Blood flow [ml/min]							Blood flow [ml/min]		
Animal number	Gender	Intact left CCA	Intact right CCA	Anastomosis	Control	Anastomosis blood flow [%]	Suture time [min]	Blood loss [g]	Anastomosis	Control	Anastomosis blood flow [%]
1	M	6.1	7.0	3.3	4.7	70.2	40	3.0	2.5	4.5	55.6
2	M	9.0	9.0	3.7	5.5	67.3	75	2.1	5.0	4.8	104.2
3	F	3.7	3.8	3.6	3.9	92.3	70	1.5	4.1	6.4	64.1
4	F	6.1	6.2	2.6	4.3	60.5	60	1.5	2.7	2.5	108.0
5	M	5.8	5.9	4.7	5.8	81.0	60	1.2	7.5	8.0	93.8
6	M	4.4	4.5	4.0	4.0	100.0	60	1.8	4.9	5.1	96.1
7	M	6.8	6.3	4.6	4.4	104.5	55	1.4	6.9	4.5	153.3
8	M	5.9	5.6	2.3	6.0	38.3	50	2.0	3.9	5.2	75.0
9	F	5.5	5.6	3.2	3.6	88.9	22	1.5	8.5	7.0	121.4
10	F	3.4	3.8	2.8	4.8	58.3	30	1.0	2.1	2.2	95.5
11	F	4.2	4.3	1.4	3.2	43.8	59	2.3	4.7	4.9	95.9
12	F	3.8	4.1	2.3	2.3	100.0	25	3.0	3.5	3.5	100.0
13	F	5.6	5.1	3.9	3.9	100.0	33	0.5	4.9	4.9	100.0
14	F	4.1	4.1	2.6	2.6	100.0	27	0.5	3.3	3.3	100.0
15	F	4.4	4.4	1.6	2.7	59.3	41	2.7	5.0	4.0	125.0
16	F	5.0	5.0	3.5	3.4	102.9	35	2.0	4.0	4.6	87.0
17	F	4.2	4.2	2.9	2.9	100.0	46	1.0	5.5	3.8	144.7
18	M	5.8	5.7	3.1	4.1	75.6	45	3.5	5.2	5.7	91.2
19	F	2.6	2.6	2.2	2.2	100.0	60	1.0	3.3	3.7	89.2

Table 2
Continuous running suture group

		First surgery							Second surgery		
		Blood flow [ml/min]							Blood flow [ml/min]		
Animal number	Gender	Intact left CCA	Intact right CCA	Anastomosis	Control	Anastomosis blood flow [%]	Suture time [min]	Blood loss [g]	Anastomosis	Control	Anastomosis blood flow [%]
1	F	4.1	4.2	3.3	2.8	117.9	22	1.0	2.6	2.3	113.0
2	F	4.7	4.7	2.8	3.7	75.7	40	1.0	3.3	3.5	94.3
3	F	2.8	2.8	2.9	3.0	96.7	35	0.5	4.8	4.8	100.0
4	F	5.0	4.8	2.8	2.7	103.7	20	4.0	6.4	5.9	108.5
5	F	3.9	3.9	3.2	3.2	100.0	25	0.5	3.1	3.1	100.0
6	F	4.7	4.7	3.1	6.1	50.8	30	0.5	5.7	4.2	135.7
7	F	5.7	5.4	2.6	5.1	51.0	25	1.0	3.3	5.8	56.9
8	M	4.9	4.9	4.6	4.8	95.8	35	0.0	3.2	3.5	91.4
9	M	5.5	5.5	5.3	6.0	88.3	23	0.5	6.1	7.0	87.1
10	M	3.6	3.6	3.4	4.0	85.0	25	0.0	4.6	3.9	117.9
11	M	3.2	3.2	2.8	2.9	96.6	35	2.0	4.3	4.7	91.5
12	M	5.3	5.2	2.7	3.6	75.0	30	1.0	6.2	5.3	117.0
13	M	5.2	5.3	2.8	3.7	75.7	35	1.0	3.7	4.5	82.2

At the beginning of the study, pilot experiments were conducted on the total of 24 animals to test various technical methodologies.

Only surviving animals with a patent anastomosis were included in the study.

Anaesthesia and analgesia

Surgery was performed under general anaesthesia. A freshly prepared anaesthetic mixture was in a syringe by mixing medetomidine (0.1 mg/kg; NarcoStart®, Produlab Pharma B.V., Netherlands), propofol (100 mg/kg; Propofol 2%, Fresenius Kabi, Germany), and nalbuphine (0.1 mg/kg; Nalbuphin Orpha, Orpha-Devel Handels und Vertriebs GmbH, Austria). Medetomidine and nalbuphine were diluted beforehand using sterile 0.9.% NaCl solution, in order to obtain working solutions of 0.1 mg/ml. The anaesthetized animal was placed on a tempered operating table and continually supplied with oxygen via a face mask. The pulse oximeter probe was attached to the hind paw or to the proximal part of the tail. Corneal reflexes, reactions to painful stimuli, pulse rate, oxygen saturation, depth and regularity of breathing were all continuously monitored. Animals were administered a reduced dose of anaesthetics (25% of the initial dose) every 40 minutes until the surgery was completed. The anaesthesia was then terminated by intramuscular application of atipamezole (0.5 mg/kg; NarcoStop®, Produlab Pharma B.V., Netherlands). The animals were then observed for the first 24 hours and received multimodal analgesia via tramadol (10 mg/kg) and carprofen (5 mg/kg) based on Zegre Cannon et al.[4] for the following 3 days. At the end of the second surgery (day 14), the animal was euthanized by an intracardial injection of potassium chloride under the general anaesthesia.

Experimental surgery

The entire surgery was performed using the surgical Zeiss OPMI CS NC-2 microscope (Zeiss Germany). Gentle microsurgical technique with high magnification was utilized.

In the supine position, a vertical straight-line midline incision over the neck was utilized to expose both carotid arteries in their maximal possible course (Figure 2). Structures were dissected along their anatomical margins to minimize bleeding and pain. Blood flow in both CCAs was measured with as little delay as possible.

In the first surgery the CCA blood flow was measured. Obtained values were recorded both in absolute number and in percentage of the blood flow of the contralateral intact CCA. The transit time flowmetry device – Transonic TS420 Flowmeter Module with the Transonic PR series TTF 1.5 mm probe (Transonic Systems Inc., USA), was used for blood flow measurement[5]. After the measurement, end-to-end anastomosis was performed. The artery chosen as surgical was clamped by a dual approximation clamp and cut transversally. After both artery stumps preparation, they were sutured together using Ethicon Ethilon 10-0 suture with a 3.8 mm needle.

In the interrupted suture group, Carrel's triangulation technique was employed[6]. It was necessary to use 14 – 18 stitches depending on the size of the vessel to minimize the anastomosis leaking (Figure 3A). In the CRS group, two sutures were used, originating at the 3rd and 9th hour positions respectively. After suturing the back wall of the vessel, the end of the first suture was tied to the first knot of the second suture. The front wall anastomosis was completed with the second suture, which was tied to the first knot of the first suture (Figure 3B).

After finishing the anastomosis, clamps were removed. In a case of significant anastomosis bleeding, additional stitches were added. Based on the findings of the pilot study, the rats tolerated very little blood loss. Blood loss of more than 3 ml (circa 12% of animal's blood volume) caused significant hemodynamic instability. Blood loss of more than 5 ml (circa 20% of animal's blood volume) was shown to be lethal. Anastomosis leaks were the only significant causes of bleeding during the surgery.

After the clip removal, blood flow measurements were performed in the same manner as before the anastomosis execution. The wound was closed in two layers afterwards. Blood loss during the surgery was determined by the weight of used cottonoids.

A second surgery was performed 14 days later, in order to measure blood flow of both CCAs. Obtained values were recorded in the same manner as during the first surgery. The anastomosis was then excised for histological examination and the animal was euthanized by an intracardial injection of potassium chloride under the general anaesthesia.

Histological evaluation

For the histological examination, the samples were submerged in Tissue Freezing Medium (Leica, Germany) and frozen at 80°C. Afterwards, the samples were unfrozen and fixed by formalin, dehydrated and embedded in paraffin blocks. The blocks were cut to 5 µm thick histological sections at the site of anastomosis as well as a more remote site. The sections were stained with haematoxylin-eosin, Verhoeff's haematoxylin and green trichrome to visualize connective tissue. Picrosirius red staining (Direct Red 80, Sigma Aldrich, Munich, Germany) was used to visualize type I and III collagen, using circularly polarized light (Figure 4). Orcein staining was used to visualize elastin. The sections were also processed immunohistochemically with anti-smooth a muscle actin antibody (dilution 1:1000, clone 1A4, Agilent Technologies, US at 4°C, overnight) to visualize the smooth muscle cells (SMC) and counterstained with Gill's haematoxylin.

Data evaluation

Student's t-test for paired samples was used to compare blood flow through intact carotid arteries at the beginning of the experiment. Normality test (Kolmogorov-Smirnov test) showed normal distribution in the interrupted suture group and abnormal distribution in the CRS group. Blood flow change between the first and the second surgery was analysed by t-test for the interrupted suture group and by Wilcoxon test in the CRS group. StatSoft STATISTICA software was used for statistical analysis.

Ethics Approval

The study was approved by the Animal Welfare Advisory Committee at the Faculty of Medicine in Pilsen and by the Ministry of Education, Youth and Sports of the Czech Republic

(approval ID: MSMT-10669/2016-3 and MSMT-33242/2018-5).

All experiments were performed in accordance with relevant guidelines and regulations.

The reporting in the manuscript follows the recommendations in the ARRIVE guidelines.

Results

The blood flow in both CCAs at the beginning of the experiment was not statistically different in all animals. The average blood flows in the right and left CCA were 4.844 ± 1.28 ml and 4.856 ± 1.26 ml respectively, and the hypothesis that flow rates are different was rejected (p =.004). The intact CCA was therefore used as a control for blood flow of the sutured CCA.

In the interrupted suture group, the anastomosis blood flow values immediately after completion of the suture ranged from 38.3–104.5% of the control CCA blood flow, with a median of 88.9% and mean average of 81.2%. The anastomosis blood flow was restricted (p =.002). Surgery time ranged from 22 to 70 minutes, with a median of 46 minutes and a mean average of 47 minutes. The length of surgery did not corelate with anastomosis flow restriction. After two weeks, the anastomosis blood flow ranged from 55.6–144.7% of the control CCA blood flow, with a median of 96.1%, and a mean average of 100.0%. The blood flow increase was present, but was statistically insignificant (p=.073). Histological examination did not show any thrombosis within the artery lumen. The inner vessel diameter (ID) at the site of the anastomosis was approximately the same as the ID of the same vessel outside the anastomosis site. Histological findings suggested that scar formation was complete in all cases (Figure 4).

In the continuous running suture anastomosis group, the anastomosis blood flow values immediately after completion of the suture ranged from 50.8–117.9% of the control CCA blood flow, with a median of 88.3% and a mean average of 85.5%. The anastomosis blood flow was restricted (p

=.025). Surgery time ranged from 22 to 40 minutes, with a median of 30 minutes and a mean average 29.2 minutes. The length of the surgery did not corelate with anastomosis flow restriction. After two weeks, the anastomosis blood flow ranged from 56.9–135.7% of the control CCA blood flow, with a median of 100.0% and mean average of 99.7%. The blood flow increase was statistically significant (p=.011). Histological examination did not show any thrombosis within the artery lumen. The inner vessel diameter (ID) at the site of the anastomosis was approximately the same as the ID of the same vessel outside the anastomosis site. Histological findings suggested that scar formation was complete in all cases.

The amount of blood loss did not affect surgical time nor blood flow through the anastomosis.

In both groups, no vasospasms were noted during the surgeries. The time required to create a fully functional anastomosis was significantly shorter in the case of CRS, compared to the interrupted suture technique (p <.001), with an average 34,8% time reduction in CRS cases. The anastomosis maturation rate after two weeks was almost identical in both groups (Figure 5).

Discussion

Brain revascularization surgeries, replantations in cases of limb loss resulting injuries or free flap procedures in reconstructive surgery all carry a risk of ischemic tissue damage. Time is therefore a limiting factor in the outcome of these surgeries[2]. Thus, time saving achieved by the use of CRS technique can be of a clinical significance in some cases. Various experiences with both suture techniques have been published. Some authors do not demonstrated significant differences between the two techniques[7], some favor the interrupted suture[8] and some favor the CRS[9]. Due to the fact, that results of these studies are ambiguous and contradictory, our experiment aimed to compare both suture techniques using the same methodological conditions, via a clinically relevant animal model.

Obtained data showed that the use of the CRS reduced time required to complete the anastomosis by 34,8%. This is supported by Moscona et al, who demonstrated time efficiency of up to 50%[10]. Adani et al. published 100% patency rate in a direct CRS end-to-end microanastomosis[11], which is supported by our study, as the patency rate after 2 weeks was 100% for both interrupted and CRS group. No maturation restriction in the CRS group was noted in our study, compared to the interrupted suture group. Both groups showed a similar blood flow increase pattern in the course of two weeks. CRS microanastomosis maturation is probably enabled by elasticity of the vessel wall and a sufficient abundance of the suture length.

The main disadvantage of CRS is that management of error repairs is more difficult and time-consuming. This disadvantage can potentially negate the time saved using the CRS technique.

Cardiac output, blood viscosity and vessel size contribute to immediate blood flow through a vessel. In our study, both CCAs showed no difference in the blood flow before performing the anastomosis. The ability to compare blood flow through the intact CCA to the operated CCA with no time delay eliminates the need to consider circulatory parameters. Obtained anastomosis blood flow values were also expressed in percentage of the intact vessel blood flow value, thus direct comparison between immediate and delayed (after 2 weeks) anastomosis blood flow was possible. Circulatory parameters during both of the surgeries can therefore be disregarded.

The scar at the site of the anastomosis was considered mature at the time of the second surgery, two weeks after performing the anastomosis. Collagen types were histologically assessed in the anastomosis scar. Presence of type I collagen was consistent with final stages of scar healing (Figure 4), suggesting 14 days interval between the surgeries was sufficient.

There was no intraluminal thrombosis in the anastomosis site. No specimens with low degree of maturation of the anastomosis after 14 days were associated with abnormal histological finding.

End-to-side microvascular anastomosis is usually used in neurosurgery when performing extra-intracranial bypass. End-to-side CCA (or ICA) to CCA microanastomosis carried high mortality in the pilot experiment preceding this study. In surviving animals, the need of transsection of the sternohyoid muscle needed for such anastomosis was too invasive and required high doses of analgesics. End-to-end microanastomosis was chosen for the experiment, because it provided the opportunity to perform delayed measurement and to precisely compare blood flow with the intact contralateral CCA. The authors are confident, that the end-to-end anastomosis model provides sufficient insight into the mechanical properties of microvascular anastomosis.

Limitations

The rat did tolerate only a very small blood loss. Any blood leakage from the anastomosis site had to be repaired by additional interrupted sutures. In order to minimize the blood loss and to minimize the risk of restricting the blood flow of the anastomosis by these stitches, the vessel had to be clamped for the whole time of the repair. This resulted in long operating times and larger time variance.

Conclusions

In our study, both CRS and the interrupted suture technique allowed microanastomosis maturation in the course of two weeks. Maturation rate in both groups was comparable. The resultant time efficiency of CRS was significant and might potentially be important in a clinical setting.

Declarations

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AUTHORS' CONTRIBUTION

JD: Performed the surgeries. Participated in the designing of the study. Discussed the results, prepared the figures and wrote the manuscript.

PK: Performed the anaesthesia and provided the postoperative care for the animals. Participated in the designing of the study. Performed the statistical analysis. Discussed the results and contributed to the final manuscript.

TB: Performed the histological examination. Discussed the results and contributed to the final manuscript.

VP: Participated in the designing of the study. Discussed the results and contributed to the final manuscript. Supervised the execution of the study.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this manuscript.

ADDITIONAL INFORMATION

The authors declare that they have no conflict of interest.

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References

1. Barros, R. S. M. *et al.* Continuous versus interrupted suture technique in microvascular anastomosis in rats. Acta Cir Bras **32**, 691–696, doi:10.1590/s0102-865020170090000001 (2017).
2. Langer, V. Management of major limb injuries. *ScientificWorldJournal* 2014, 640430, doi:10.1155/2014/640430 (2014).
3. Mikami, T. *et al.* Surgical Anatomy of Rats for the Training of Microvascular Anastomosis. World Neurosurg **120**, e1310-e1318, doi:10.1016/j.wneu.2018.09.071 (2018).
4. Zegre Cannon, C., Kissling, G. E., Goulding, D. R., King-Herbert, A. P. & Blankenship-Paris, T. Analgesic effects of tramadol, carprofen or multimodal analgesia in rats undergoing ventral laparotomy. Lab Anim (NY) **40**, 85–93, doi:10.1038/laban0311-85 (2011).
5. Charbel, F. T., Hoffman, W. E., Misra, M. & Ostergren, L. Ultrasonic perivascular flow probe: technique and application in neurosurgery. Neurol Res **20**, 439–442, doi:10.1080/01616412.1998.11740545 (1998).
6. Aida, L. Alexis Carrel (1873-1944): visionary vascular surgeon and pioneer in organ transplantation. J Med Biogr **22**, 172–175, doi:10.1177/0967772013516899 (2014).
7. Alghoul, M. S. *et al.* From simple interrupted to complex spiral: a systematic review of various suture techniques for microvascular anastomoses. Microsurgery **31**, 72–80, doi:10.1002/micr.20813 (2011).
8. Radad, K. & El-Shazly, M. Clinical and pathological assessment of different suture techniques for microvascular anastomosis in rat femoral artery. J Vet Sci **8**, 269–273, doi:10.4142/jvs.2007.8.3.269 (2007).
9. Chen, Y. X., Chen, L. E., Seaber, A. V. & Urbaniak, J. R. Comparison of continuous and interrupted suture techniques in microvascular anastomosis. J Hand Surg Am **26**, 530–539, doi:10.1053/jhsu.2001.22933 (2001).
10. Moscona, A. R. & Owen, E. R. Continuous anastomotic technique in microsurgery. Isr J Med Sci **14**, 979–983 (1978).

11. Adani, A., Castagnetti, C., Lagana, A., Perretti, M. & Caroli, A. Proposition for a new continuous suturing technique for microvascular anastomosis: a comparative study. Br J Plast Surg **41**, 506–508, doi:10.1016/0007-1226(88)90008-2 (1988).

Figures

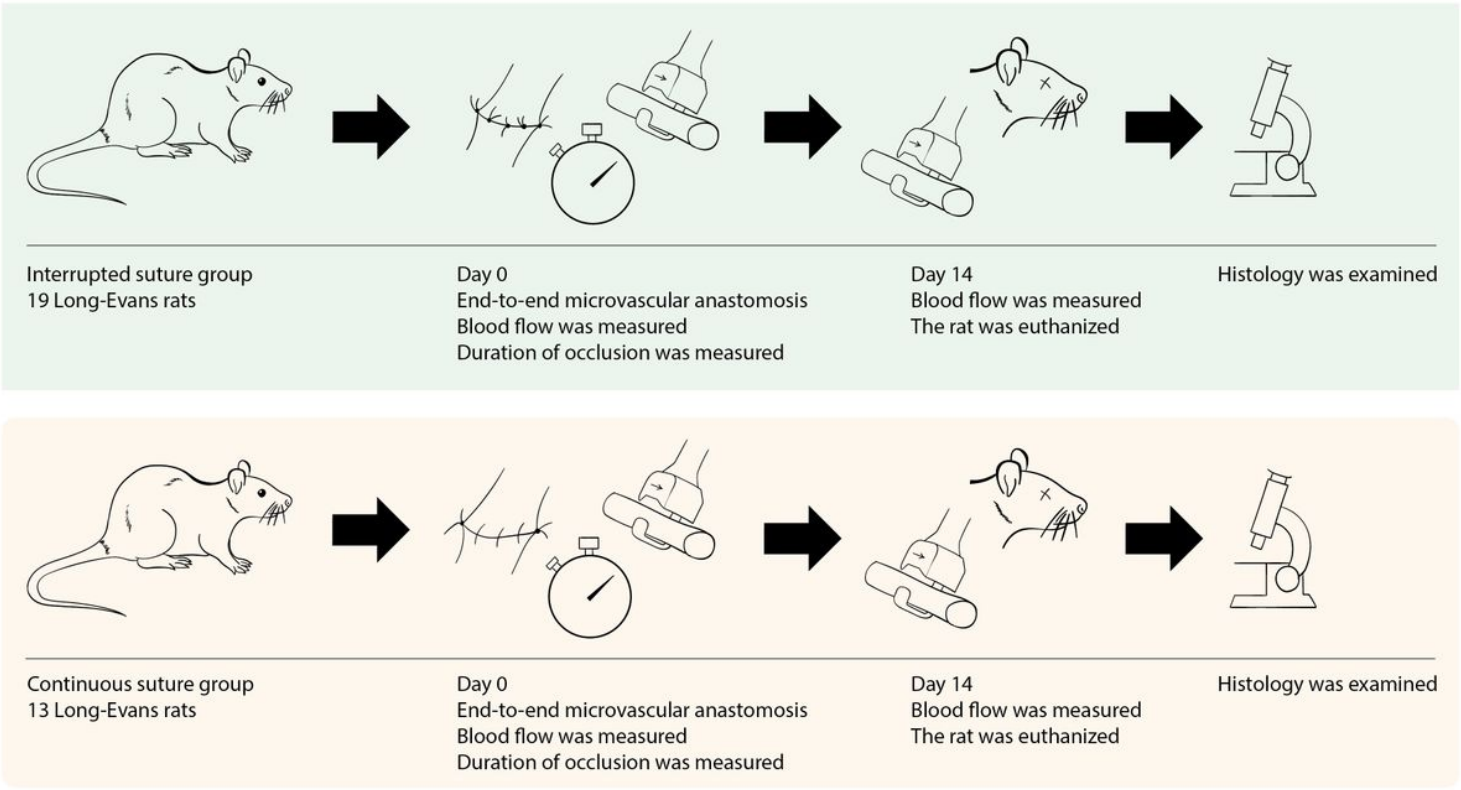

Figure 1

Graphical representation of the methods of the study

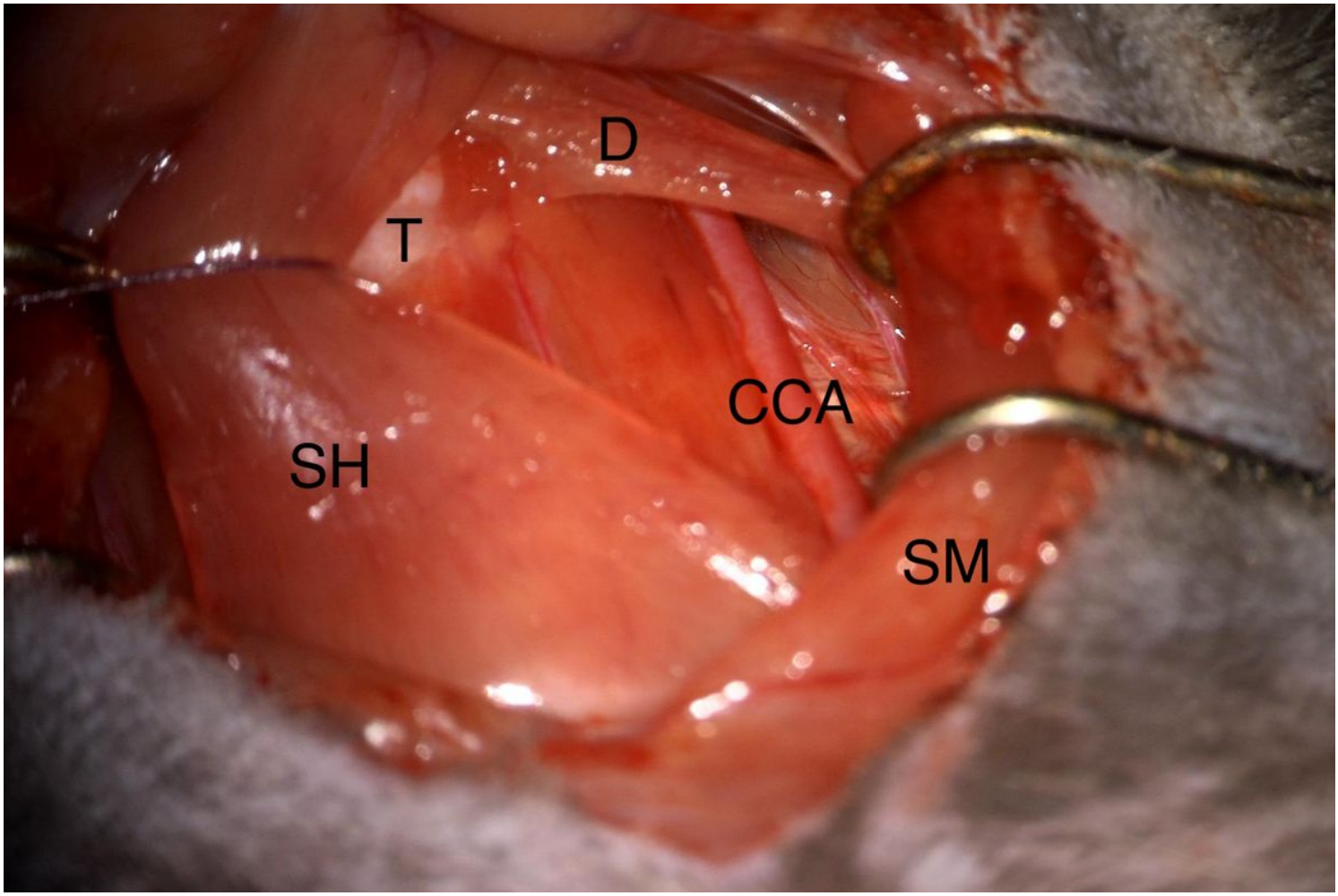

Figure 2

Exposed left CCA before the microanastomosis. SH – Sternohyoid muscle, T – Trachea, D – Digastric muscle, CCA – Common carotid artery, SM – Sternomastoid muscle

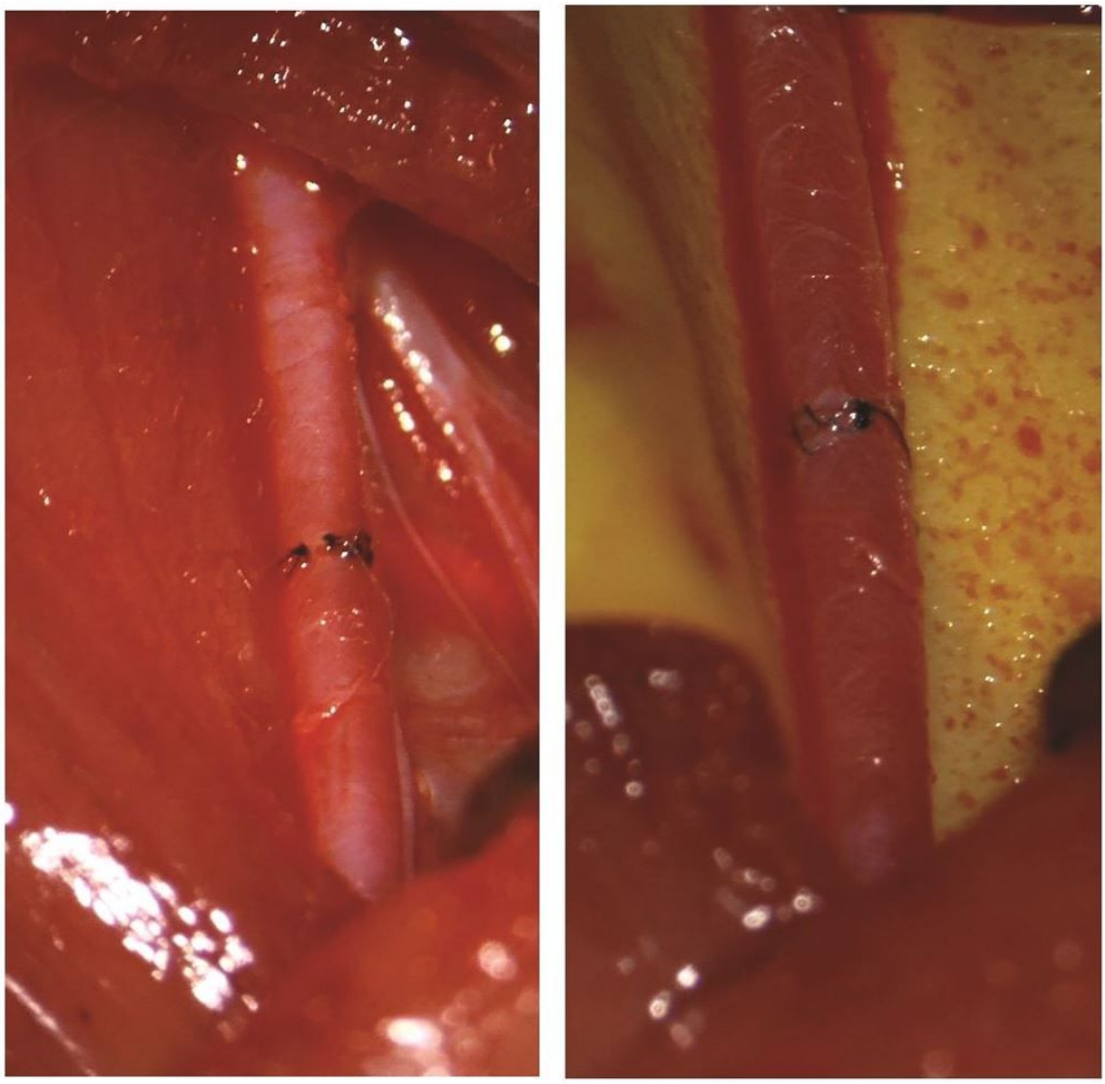

Figure 3

3A: Complete interrupted suture microanastomosis

3B: Complete continuous running suture microanastomosis

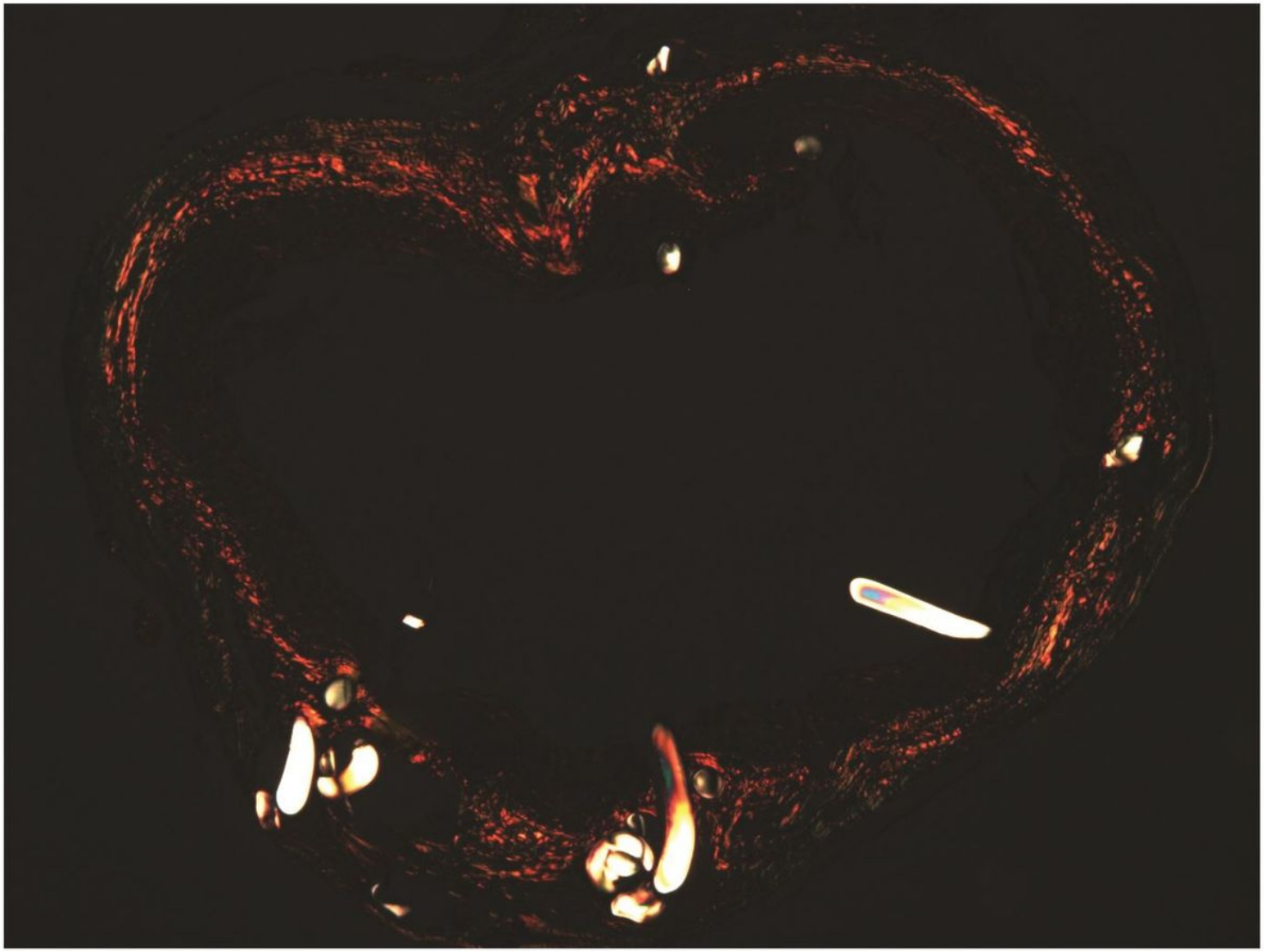

Figure 4

Mature strong type I collagen (red) was present in the whole arterial wall and around sutures (white). Picrosirius red staining in polarized light was used.

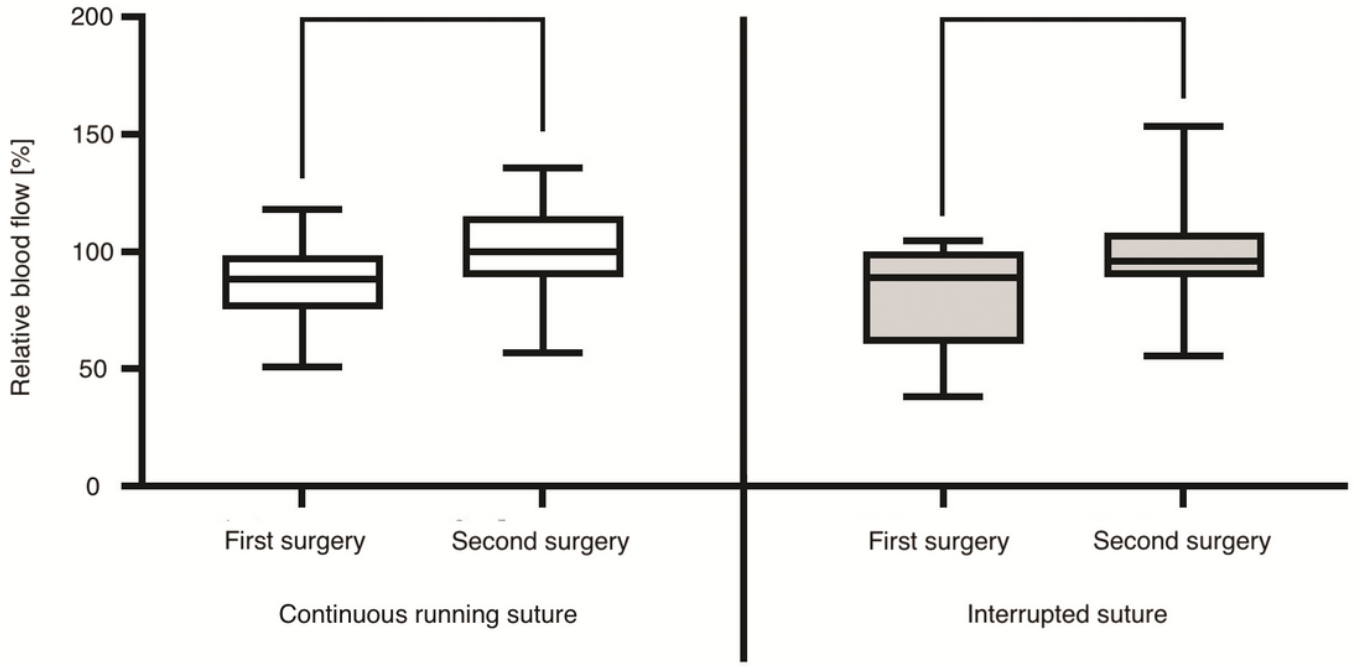

Figure 5

Boxplot showing minimal, maximal and median blood flow values through the anastomosis.