Original Article

# Immediate intermittent compression over vascular anastomosis: a new technique to prevent thrombosis?

## *Compressão intermitente imediata sobre anastomose vascular: Uma nova técnica para prevenir trombose?*

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■ **ABSTRACT**

**Introduction:** This study aims to evaluate the effect of immediate intermittent compression on microsurgical arterial anastomoses in comparison with fixed compression and only observation in an experimental laboratory. **Methods:** The two femoral arteries of twelve male Wistar rats were sectioned and reanastomosed to compare patency at 30 minutes and 7 days. Group I: immediate intermittent compression was performed over the anastomosis for 60 s; group II: a fixed compression was maintained immediately after the anastomosis for 60 s; group III: after completion of the anastomosis, no additional intervention was performed. In addition to the patency assessment, the animals were weighed and the operated arterial diameters were measured. **Results:** Twenty-four femoral arteries were examined. Initial average weights of the rats in groups I, II, and III were 243.8g, 254.6g, and 260.4g, respectively, while the final weights were 264.4g, 281g, and 282.1g ($p<0.001$), respectively; mean diameter of the approached arteries was 0.89, 0.88, and 0.90mm, respectively, and the anastomoses (time in minutes) were 25.6, 24.5, and 24.5, respectively; final patencies after 7 days were 62.5% ($p=0.07$), 25% ($p=0.48$), and 50% ($p=0.13$), respectively. **Conclusion:** Immediate intermittent compression can be performed at the end of microsurgical arterial anastomoses without affecting the final patency of the procedure.

**Keywords:** Arteriovenous anastomosis; Microsurgery; Plastic surgery procedures; Thrombosis; Rats, Wistar.

■ **RESUMO**

**Introdução:** Este estudo tem o objetivo de avaliar o efeito da compressão intermitente imediata sobre anastomoses arteriais microcirúrgicas em comparação com compressão fixa e com utilização isolada de irrigação com soro fisiológico e heparina em laboratório experimental. **Método:** 12 ratos Wistar foram aleatoriamente divididos em três grupos para terem suas artérias femorais seccionas e anastomosadas de forma término-terminal, para comparação de patência com 30 minutos e 7 dias. Grupo I: foi realizada compressão intermitente imediata sobre a anastomose por 60 segundos; grupo II: uma compressão fixa foi mantida imediatamente após a anastomose, também por 60 segundos; grupo III, após o término da anastomose, não foi feita nenhuma intervenção adicional. Além da avaliação da patência, os animais foram pesados e medidos os diâmetros arteriais operados. **Resultados:** 24 artérias femorais foram abordadas. As médias de peso inicial dos ratos dos grupos I, II e III foram, respectivamente, de 243,8g, 254,6g e 260,4g, enquanto as finais foram de 264,4g, 281g e 282,1g ($p<0,001$). O diâmetro médio das artérias abordadas foi, respectivamente, de 0,89mm, 0,88mm e 0,90mm, e os tempos de anastomoses em minutos, de 25,6, 24,5 e 24,5, respectivamente; As patências finais após 7 dias foram, respectivamente, de 62,5% ($p=0,07$), 25% ($p=0,48$) e 50% ($p=0,13$). **Conclusão:** A compressão intermitente imediata pode ser realizada ao término de anastomoses arteriais microcirúrgicos sem prejuízo na patência final do procedimento.

**Descritores:** Anastomose arteriovenosa; Microcirurgia; Procedimentos de cirurgia plástica; Trombose; Ratos Wistar.

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Article received: November 22, 2023.
Article accepted: February 4, 2024.

Conflicts of interest: none.

**DOI: 10.5935/2177-1235.2024RBCP0905-EN**

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## INTRODUCTION

Advances in optical magnification techniques have allowed surgeons to approach smaller structures, creating a huge range of possibilities for different specialties. This set of procedures aided by optical magnification is known as microsurgery[1].

Soon after the dissemination of the techniques, it was realized that a properly trained surgeon[2] was a fundamental component. Thus, the first training laboratories soon appeared, Brazil being a pioneer in South America, in 1971[3].

The training sequence varies a little between the various current centers and generally starts with familiarization with the microscope and other materials, followed by learning variations of microsurgical sutures, with simple materials such as latex gloves[4], until evolving to vascular anastomoses in non-living and living models, when available[5].

The training aims to develop the professional skills of the surgeon, to prepare and provide security during his clinical activities; excellent results are anticipated in vascular anastomoses patency, since the primary services report flap survival rates above 90%[6,7].

Non-biological models avoid ethical conflicts related to the use of animals, in addition to the fact that some are accessible, such as tubes made of synthetic materials, and others allow a more accurate assessment of skills, particularly virtual ones; however, animal models are more consistent and closer to the reality found in clinical practice[8-10].

One of the techniques necessary for training in microsurgery is vascular anastomosis. Some fundamental rules of vascular anastomoses should be followed, which include well-vascularized proximal and distal margins, free of active disease and tension. The anastomosis also should be hemostatic, circumferentially air-tight, and have inverted edges[11].

Thrombosis being one of the main obstacles to the success of vascular flow, specific animal models have been developed to assess the impact of endothelial damage on thrombosis formation [12], demonstrating how aggression to the intimal layer can evolve into this complication and how external applications of different forces can cause similar consequences [13]. Even though the concept of compression anastomosis has been investigated for nearly two centuries, it has not yet achieved widespread acceptance, but it could be a way of reducing the risk of thrombosis[11].

It is known that after arterial thrombosis of the anastomosis, the flap may survive with only conservative therapy when the event occurs after a minimal critical period. This time is most likely to occur after the 12th postoperative day, but this minimum critical period may be as low as 6 days for arterial occlusion[14]. Thus, it is essential to comprehend and develop ways to avoid thrombosis, when feasible, and treat it, when necessary, for successfully performing complex microsurgical flaps.

## OBJECTIVE

This study primarily aimed to evaluate the impact of immediate intermittent compression on microsurgical arterial anastomoses and their patency in an experimental model. The secondary objective was to evaluate the impact of gentle fixed compression immediately after vascular clamp removal.

## METHODS

During experimental training, 12 Wistar rats (male sex; age, 60–90 days) were selected for sectioning and anastomoses of the two femoral arteries, to assess the impact of immediate intermittent compression on microsurgical arterial anastomoses. All animals received bilateral anastomosis by the same method.

The study was carried out between April and July 2021 and the CEP approval number is 1378/2021.

The 24 arterial anastomoses were randomly divided into three groups, as described below. A draw was performed immediately before the anesthesia of the rats, and a paper written with the technique to be performed on each rat was removed from the bag.

Group 1 (G1) – Intermittent Compression for 60 s Group 2 (G2) – Fixed Compression for 60 s Group 3 (G3) – Control (observation only)

In Group 1, immediately after microsurgical anastomosis, 60 gentle intermittent compressions were performed, such as massage, for 60 seconds (Figure 1). In Group 2, the same gentle compression was applied constantly, for 60 seconds. In Group 3, anastomosis was only observed without any intervention. The measurement of the diameter of the vessels was performed with a millimeter ruler under a microscopic view with a 10x magnification before the procedure.

### Schedule

The study was divided into two intervention periods. The first period involved a random division of the femoral arteries to separate the groups and perform the initial surgery. In the second period, after 7 days, the final patency of the anastomosis was checked.

Initial data included weight, vessel diameter at the section point, surgical time for end-to-end arterial anastomosis, and the number of sutures. The final data included weight and patency assessments after 7 days.

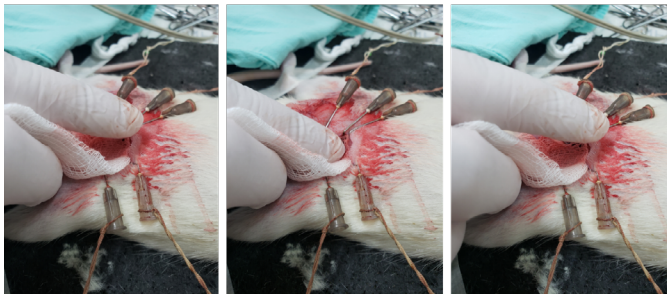

**Figure 1.** Intermittent compression applied over the newly performed arterial anastomosis.

### Anesthesia

We opted for combined anesthesia using 10% ketamine hydrochloride (Cetamin®, Syntec, Brazil) and 2% xylazine hydrochloride (Xilazin®, Syntec, Brazil) intraperitoneally, at doses of 80 and 10 mg/kg, respectively, associated with lidocaine (Xylestesin®, Cristália, Brazil) for application at the incision sites at a dose of 7 mg/kg. For intra-and postoperative analgesia, tramadol (Tramadon®, Cristália, Brazil) and dipyrone (Febrax®, Lema-Injex Biologic, Brazil) were subcutaneously administered at doses of 5 mg/kg and 100 mg/kg, respectively. Dipyrone was reapplied daily for pain control until the second procedure and subsequent euthanasia of the animal with thiopental (Thiopentax®, Cristália, Brazil), at a dose of 120 mg/kg, on the seventh day.

### Surgery

After initial weighing and trichotomy of the bilateral inguinal region, an oblique incision was made in the inguinal region of the rat, with opening and dissection by plane until the identification of the femoral vessels. The femoral artery was dissected and isolated at its most proximal and largest caliber, with a blue ribbon under it for better contrast. At this point, the adventitial layer was removed, and the diameter of the artery was measured at the point where it was sectioned. Before the section, the double metallic clamp was positioned proximally and distally to this point. An interrupted suturing was initiated using 10-0 mono nylon, with 3/8 and 0.65 cm cylindrical needles to perform the anastomosis. After the end-to-end suture was timed and the number of sutures was counted, immediately after clamp removal, one of the three proposals was performed, according to the group chosen for the artery. We allowed 30 min to check the patency, as described below and then proceeded to the approximation of the muscle, subcutaneous tissue, and skin of the rat using 5-0 mono nylon simple sutures.

After 7 days, the rats were anesthetized again. After weight measurement, the vascular suture was reexplored, and patency was checked.

### Patency test

Patency was always verified by an independent examiner.

Patency at 30 min and 72 h was verified using the technique proposed by Acland[13]. Two forceps were placed distally to the anastomosis, and a maneuver was performed to empty the central region at both sites. Subsequently, the proximal forceps were released to refill the central space. If the created intravascular space was immediately filled with blood proximal to the distal direction, we considered the patency (flow) to be successful.

Vessel staining and pulsation were considered indirect signs of successful patency (flow).

The absence of flow after 30 min or 7 days was considered an anastomosis failure. The limitation of this test is that although it is useful to verify the technique, it does not consider the blood flow.

### Statistical analysis

The data were tabulated using Microsoft Excel software. Profile analysis was used to determine the initial and final weights (Table 1).

Analysis of variance was used for other quantitative variables (Table 2).

For patencies in the initial and final moments, the McNemar test was used (Table 3).

### Experimental Laboratory

All activities were performed in an experimental laboratory at the University of Medicine of Botucatu, where bioterium, basic microsurgical material (Microsuture®), and microscopes (DFV®) with magnification up to 40 times are available.

**Table 1.** Mean and standard deviation for mass according to the moment.

| | Mass (g) | |
|---|---|---|
| | Initial | Final |
| G1 | 243.8 | 264.4 |
| | 38.2 | 22.2 |
| G2 | 254.6 | 281.0 |
| | 39.5 | 42.2 |
| G3 | 260.4 | 282.1 |
| | 33.5 | 18.7 |

**Table 2.** Mean and standard deviation for variables according to the group.

| | Variables | | |
|---|---|---|---|
| | Artery diameter (mm) | Number of sutures | Time (min) |
| G1 | 0.89 | 6.5 | 25.6 |
| | 0.04 | 1.4 | 2.0 |
| G2 | 0.88 | 7.0 | 24.5 |
| | 0.05 | 0.8 | 3.7 |
| G3 | 0.90 | 6.0 | 24.5 |
| | 0.08 | 1.1 | 3.9 |
| *p* | 0.67 | 0.22 | 0.74 |

**Table 3.** Absolute and relative frequency of initial and final patency according to the group.

| | Patency | | |
|---|---|---|---|
| | Initial | Final | *p* |
| G1 | 8 | 5 | 0.07 |
| | 100.0 | 62.5 | |
| G2 | 8 | 2 | 0.48 |
| | 100.0 | 25.0 | |
| G3 | 8 | 4 | 0.23 |
| | 100.0 | 50.0 | |
| Total | 24 | 11 | |
| | 100.0 | 45.8 | |

Animals were kept in different cages, with water and food ad libitum, an air exhaust system, 12 h of light/dark cycles, and temperature control, in addition to materials for environmental enrichment.

At the end of the procedure, the rats were sacrificed, and respective carcasses were kept frozen at -20 °C until further use for experimental training. When necessary, the carcasses were incinerated.

### Animal Use Ethics Commission (AUEC)

This research was conducted according to the rules of the AUEC of the University of Medicine of Botucatu and was approved by the same under registration number 1378/2020.

## RESULTS

Twenty-four anastomoses were performed in the 12 rats included in the study, which were divided into three groups, with 8 vascular anastomoses in each group. Initially, the weight of the animals was evaluated before performing the procedure and, after the surgery, the rats showed weight gain in all three groups ($p < 0.001$), as can be seen in Figure 2. The average of the individual weight values of the rats in each group can be seen in Table 1, however, there was no statistically significant difference in weight when comparing the three groups ($p = 0.53$), nor when comparing weights before and after the procedure ($p = 0.84$).

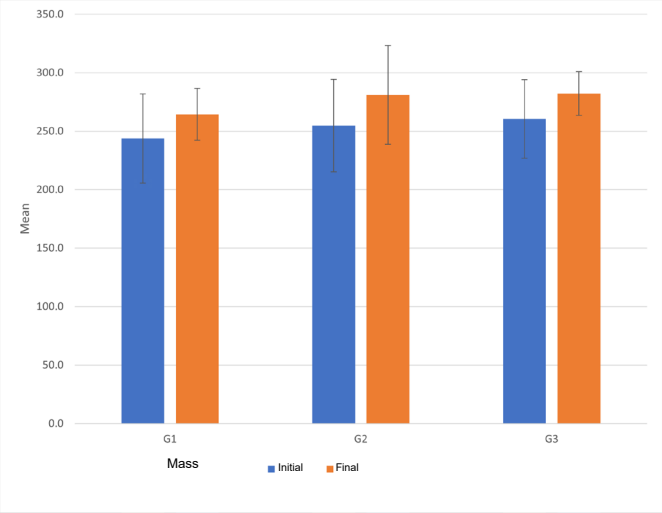


**Figure 2.** Mean and standard deviation for mass according to the moment.

Another quantitative variable evaluated before the procedure was the measurement of the diameter of the artery to be anastomosed, as can be seen in Figure 3 ($p = 0.67$), and the number of sutures performed in each group, as can be seen in Figure 4 ($p = 0.22$). There was no statistical significance ($p > 0.05$) in terms of the frequency of patency according to the group. The mean operative time was also considered in each group, being slightly greater than 25 minutes in Group 1, which can be seen in Figure 5 ($p = 0.74$).

Regarding the patency rate, it was observed that in all groups there was a patency rate of 100% immediately after the anastomosis, but after 7 days the patency rate was 62.5% ($p = 0.07$), 25% ($p = 0.48$), and 50% ($p = 0.13$) in Groups 1, 2, and 3 respectively. Consequently, the thrombosis rate was 37.5%, 75% and 50% in groups 1, 2, and 3, respectively, highlighting that in Group 2 there was a higher rate of thrombosis in relation to the other groups with statistically significant difference ($p < 0.05$).

## DISCUSSION

The practical application of microsurgery in specialized services involves a triad of fundamental conditions: professional training, appropriate

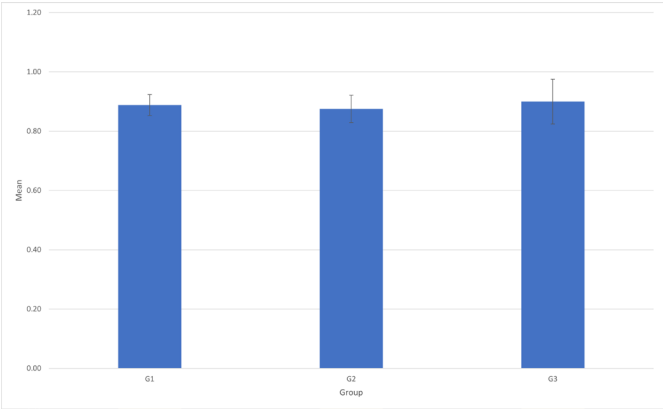


**Figure 3**. Mean and standard deviation for the artery diameter according to the group.

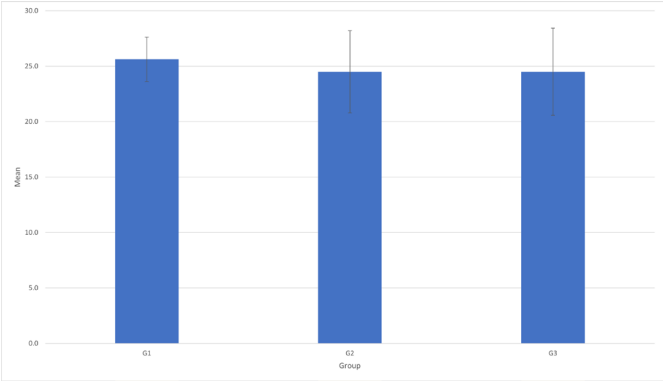


**Figure 4**. Mean and standard deviation for time according to the group.

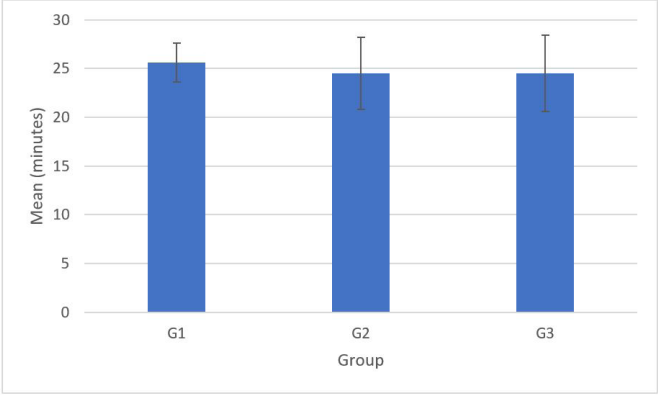


**Figure 5.** Mean and standard deviation for time according to the group.

instruments, and hospital support[3]. Through a literature review, it was not possible to identify any studies that applied any tactic similar to our research, although several studies discussed factors that would be important to prevent or treat arterial thrombosis.

It might be complex to predict which anastomosis might be damaged by thrombosis. In humans, it is crucial to consider the patient's clinical condition; however, this is more complex in animal models without specific or genetically programmed alterations. The usefulness of ultrasonography by flow measurement has already been demonstrated; however, the procedure is not viable in common laboratories[15].

Irrigation with heparin solution has already proven to be useful in the prevention; however, it has not been described as capable of causing thrombolysis[13]. Some studies have attempted to define a balanced concentration of this solution [16] and have estimated that 100 U/mL is superior to 250 U/mL. The dose used in our experience was 150 U/mL and we considered it safe, as we did not observe any abnormal bleeding.

Heparin, as well as its derivatives, including Dalteparin (a low molecular weight form of heparin), which does not increase the risk of surgical bleeding, are known to be an effective microsurgical anastomoses approach[17].

Lidocaine has a special effect on the treatment of vasospasms, with arapid onset of action, but of short duration[18]. The 2% lidocaine applied in our study had a satisfactory arterial dilation effect, concerning the action interval proposed by Ogawa et al.[19].

Several studies have evaluated the use of certain medications, including acetylsalicylic acid, parenteral heparin, and glycoprotein IIB-IIIA inhibitors (tirofiban); however, these show benefits only when administered together[20]. When heparin and tirofiban were associated with intraluminal injection before passing the last point of the anastomosis, there was a significant increase in serial patency with 62 anastomoses[21].

Some natural antioxidant flavonoids, such as epigallocatechin, which is present in green tea, were tested against saline solution on the microsurgical arterial anastomosis site with good effects; when easily accessible to the laboratory, they can be another tool to prevent early thrombosis and cause vasodilation[22].

Type III phosphodiesterase inhibitors, such as cilostazol, have already been successfully applied in animal models designed to develop arterial thrombosis, with a statistically significant success[23]. However, due to its application a few hours before surgery, it increases the preparation time for training and is not as practical as the measures presented in our series, which were all performed quickly and during the intraoperative period.

Pentoxifylline, a xanthine-derived drug with peripheral vasodilator action, has already been successfully used in experimental models that favor thrombosis[24]. However, the technique involved serial applications, which also increased the preparation time and the cost of training.

Among other possibilities, the use of 10% ethanol, applied subcutaneously, close to the vessel area, 7 days before the surgical approach, has been reported[25]. Ethanol can activate nitric oxide and prostaglandins, reduce vasospasm, and inhibit platelet aggregation.

This study reported an increase in vascular diameter and lower rates of arterial thrombosis.

Botulinum toxin also plays a role in the prevention of arterial thrombosis. Both types A[26] and B[27] show benefits by reducing thrombosis rates, in addition to generating vessels with larger diameters.

Mechanical actions also directly affect patency rates. When some type of torsion[28] or changes in the angulations[29] of the anastomoses are performed, the patency rates decrease proportionally.

When comparing the patency rate between the different techniques, the intermittent compression was superior, in absolute numbers, in relation to the others; fixed compression had the lowest absolute patency index. However, there was no statistical difference between the groups, although Group 1, intermittent compression, showed $p=0.07$. Considering that the number of rats in this study was small, a probable statistical difference could be expected if more arterial anastomoses were performed. It was not possible to find in the literature other articles that carried out studies with the use of fixed and intermittent compression to reduce thrombosis and therefore it was not possible to make a comparison with similar articles.

Most studies compare the rates of anastomosis patency in the period between one and 24 hours after the procedure. A recent study published with the use of heparin in arterial microsurgical anastomosis found a rate of 13% of thrombosis after 1 hour of anastomosis compared with the control group. Another study that compared the patency of surgical microsurgical anastomosis using sildenafil and papaverine observed an immediate thrombosis rate of 20% and 30%, respectively, compared to the control group[30]. The present article compared the groups with the patency rate at 7 days after anastomosis, which may explain the lower patency rates compared to the literature.

A fundamental tactic is the need to avoid the use of solutions or surgical environments at low temperatures since hypothermia is known to be a complicating factor, leading to vasospasm and higher rates of thrombosis.

In the future, the presented tactic can be tested in venous anastomoses, serving as advanced training since the veins of the approached animals have a small caliber, making the procedure more challenging. Further studies using a greater "n" should be conducted to validate the superiority of the intermittent compression technique.

There are some methodological limitations in the present study: the small number of animals as a consequence of the low availability in the experimental surgery, the absence of a group with heparin, and the fact that the anastomosis performed at one femoral artery may affect the contralateral anastomosis patency.

## CONCLUSION

Arterial compression proved to be a possibility in an attempt to reduce thrombosis rates in microsurgical arterial anastomosis. Although intermittent compression had lower risks of thrombosis, there was no statistically significant difference in this study. It was also observed that fixed compression increased the chances of anastomosis failure. Thus, it is necessary to carry out studies with larger populations to determine the validity of mechanical methods in arterial anastomoses.

**Acknowledgments**

We thank the professionals of the Experimental Research Units of the University of Medicine of Botucatu and professor and statistician, Lídia Raquel de Carvalho. We would like to thank Editage for English language editing.

## COLLABORATIONS

**BFMN** Analysis and/or data interpretation, Conception and design study, Conceptualization, Data Curation, Methodology, Project Administration, Realization of operations and/or trials, Writing - Original Draft Preparation, Writing - Review & Editing.

**MSF** Analysis and/or data interpretation, Data Curation, Realization of operations and/or trials.

**LVM** Analysis and/or data interpretation, Data Curation, Writing - Review & Editing.

**FV** Conceptualization, Supervision.

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