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Sutureless venous microanastomosis using thermo-sensitive poloxamer and cyanoacrylate: experimental study on a rat model

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Abstract:

Introduction: Sutureless microvascular anastomoses could simplify the microvascular field, shortening operative time and improving the final outcome. The use of thermo-sensitive poloxamers together with application of cyanoacrylate as an alternative method for conventional sutures was well documented for arteries, but not for veins. The purpose of our study was to prove the feasibility of this technique for venous anastomoses and compare it with the traditional hand-sewn technique on a rat model.

Materials and Methods: Twenty male Sprague-Dawley rats weighing between 265 and 310 g were used. In the sutureless group (SG), twenty left external jugular veins (LEJV) end-to-end anastomoses were performed using a thermo-sensitive poloxamer and cyanoacrylate glue. They were compared to twenty right external jugular veins (REJV) anastomoses sutured with conventional 10-0 stitches (control group - CG). Diameters of veins, anastomosis time and patency rate at 15 days were reported. Foreign body reaction was assessed histologically.

Results: The mean diameter of the LEJV was 0.94 ± 0.1 mm and 0.95 ± 0.09 mm for the REJV. The mean anastomosis time was 11.9 ± 1.37 minutes for the SG and 27.75 ± 3.31 minutes for the CG. In the latter group, the immediate patency rate was 95 % and 90 % at 15 days. For the SG group, 90% of the anastomoses were patent immediately and 85 % at 15 days.

Conclusion: Thermo-sensitive poloxamer and cyanoacrylate could offer a fast and reliable technique for sutureless venous anastomoses. Before human application, effectiveness of this method remains to be confirmed **in larger animals in a long-term follow-up**.

Key words: microsurgery; venous sutureless anastomosis; poloxamer; cyanoacrylate

INTRODUCTION:

Vascular anastomoses represent a key point in the field of microsurgery. Even if numerous methods have been described so far, the gold standard remains the hand-sewn technique. However, the latter one still presents some disadvantages, such as endothelial injury, suture reactions, prolonged surgery or costly education¹⁻³.

In order to overcome some of the non absorbable suture-related complications, several sutureless techniques were described. Chang et al.⁴ achieved convincing results in sutureless microanastomoses by using a thermo-reversible poloxamer 407 (P407) as a biodegradable filler, followed by application of 2-octylcyanoacrylate on a rat model. They reported long-term patency, less inflammatory cell infiltration and significantly faster procedure compared to traditional hand sutured anastomoses. Furthermore, our study published in 2015⁵ showed the reliability of this method also on a rat model, with a poloxamer that could be used at an ambient temperature (TP 407), contrary to the P 407 engineered by Chang et al.⁴.

However, despite these results, there is no evidence that this method could be applicable for venous anastomoses. Venous walls are thinner than arterial walls, with less smooth muscle and elastic tissue⁶. Therefore, venous anastomoses are more technically demanding, as veins are more susceptible to collapse during the procedure.

The purpose of this study was to prove the feasibility of the sutureless method using a thermo-sensitive poloxamer and cyanoacrylate for end-to-end venous anastomoses, and to compare the results with the traditional hand-sewn technique on a rat model.

MATERIALS AND METHODS:

Thermo-sensitive Poloxamer (TP407):

LeGoo® is comprised of a 20% (weight percent in saline) of purified poloxamer 407, a non-toxic gel which is part of a family of biocompatible, water-soluble polymers that possess reverse thermo-sensitive properties (i.e. as temperature increases, viscosity increases). LeGoo® is a nontoxic, non-absorbed and non-metabolized polymer (FDA-Summary and Effectiveness Data-PMA P110003).

When injected into the blood vessel, the viscosity increases and it maintains the vessel lumen open. This TP 407 dissolves spontaneously after few minutes in contact with blood or instantaneously after application of cold saline (less than 19°C). Once dissolved below a minimum concentration, the polymer does not resolidify and is excreted unmetabolized in the urine.

Cyanoacrylate:

We used a cyanoacrylate monomer, n-hexyl- α -cyanoacrylate (NHCA) (Ifabond™, Fimed, France), which is a very pure adhesive with low viscosity for internal and external usage (implantable medical device of marked class III CE mark). When applied in vivo, NHCA polymerizes rapidly and it provides strong fixation after 30 seconds.

Animal model:

Twenty Sprague-Dawley male rats weighing between 265 and 310 g were used. The study was approved by the local University's animal ethics committee. The animals were divided into two groups: 1) control group (CG), in which end-to-end anastomoses were

performed using interrupted 10/0 nylon sutures and 2) sutureless group (SG), where the association of Legoo® and NHCA was used for the venous anastomoses (Figure 1).

Surgical procedure:

All procedures were performed under general anesthesia, with diazepam 5 mg/kg and ketamine 100 mg/kg administered intra-peritoneal. 16x surgical magnification was used for vessels dissections and anastomoses.

The external jugular veins (EJV) were exposed through a cervical incision. Branches of both EJV were ligated with 10/0 sutures. First, the right external jugular vein (REJV) was anastomosed by the senior plastic and reconstructive surgeon using a conventional interrupted suture method (CG). Then, the left external jugular vein (LEJV, Fig. 1A) was anastomosed by the junior surgeon, who just obtained a microsurgery degree, using the association of Legoo® + NHCA (SG). After irrigating the two ends of the vessel with 0.9 per cent sodium chloride solution, surrounding connective tissue was removed and the two vessel stumps were prepared (Fig. 1B). 0.3 ml of Legoo® were injected at an ambient temperature ($> 20^{\circ}\text{C}$) (Fig. 1C) and after approximation of the stumps, 0.3 ml of NHCA were applied circumferentially using the tip of a thin needle (Fig. 1D). After allowing the glue to dry for one minute, the vascular clamp was released. The blood flow liquefies the gel, which will be excreted in the urine (Fig. 1E,F). Finally, an immediate patency test was performed. Anastomoses were considered successful when the patency test was still positive 15 minutes after the end of the procedure. In case of non-spontaneously resolute leakage, neither sutures nor glue were added and the anastomosis was considered as unsuccessful.

All data were recorded prospectively for each procedure. The weight of each rat was measured before and 15 days after surgery. Duration of microvascular anastomosis (DMA) was defined as the time from the section of the external jugular vein to unclamping. Patency

of each anastomosis was assessed two times with the O'Brien test: 15 min after unclamping and 15 days after surgery (Fig. 1.8). For each jugular vein, measurements were made with the ImageJ version 1.43u software.

For histological analysis, 5 rats from each group were sacrificed at day 15. Anastomoses of both control and sutureless groups were fixed in ten per cent neutral formalin solution, embedded in paraffin, sectioned at 4 μ m and stained with haematoxylin and eosin. Inflammatory reaction, the number of inflammatory and multinuclear giant cells at the anastomosis site was evaluated under light microscopy at **x100 magnification**.

Statistical analysis:

Statistical analysis was performed using the XLStat version 7.5.2 software. Descriptive statistics were presented as mean and standard deviation (Table 1). Shapiro-Wilk test was used to assess normality of variables. Pearson coefficient was used to assess correlation between variables. A paired samples *t*-test was used to compare both groups' data. A bilateral p-value < 0.05 was considered significant.

RESULTS:

The mean weight of rats was 287.1g (range 265-310) preoperatively and 285.45g fifteen days after the surgery (range 262-304). The mean diameter of the REJV was 0.95 ± 0.09 mm and 0.94 ± 0.1 mm for the LEJV. There was no significant difference of diameter between REJV and LEJV ($p=0.32$), ensuring comparability of both groups.

The DMA ranged from 9 to 15 minutes for the sutureless group, with an average of 11.9 ± 1.37 minutes. For the control group, the mean DMA was 27.75 ± 3.31 minutes (range 22-35) (Fig. 2). No correlation could be found between both REJV and LEJV diameters and the DMA ($p=0.80$). However, when comparing the DMA between the two groups, we observed that the anastomosis time was significantly shorter in the SG than in the CG ($p<0.0001$). The immediate patency rate in the CG was 95 % (n=19/20) and 90 % (n=18/20) at 15 days. For the SG, 90% of the anastomoses were patent at the end of the procedure and 85 % (n=17/20) at day 15. Patency rates did not differ between the groups, whether immediately ($p=0.55$) or 15 days after surgery ($p=0.63$).

Histological analysis showed moderate adventitial and media inflammation with respect of the intima (Fig. 3). Lower foreign-body giant-cell reactions were observed in perivascular location, but no significant difference between the two groups was found at day 15 post-procedure ($p=0.90$).

DISCUSSION:

Regardless of the suturing method, restoration of the cylindrical structure of the vessel ends is crucial for the success of microvascular anastomosis. Different biological and synthetic glues have been commonly used and tested as adjuncts to conventional vascular sutures^{7,8,9}. All these techniques showed promising results, but to our knowledge, sutureless venous anastomoses were reported only on a small number of animal models with a short term follow-up¹⁰.

The vessel walls of veins are thinner than arterial walls and do not have as much tunica media. The tunica media is smaller in relation to the lumen of arteries, containing less smooth muscle and elastic fibers. Therefore, venous anastomoses are more technically demanding, as veins are more susceptible to collapse during the procedure. This study demonstrates the feasibility of sutureless venous anastomoses with using a thermo-sensitive poloxamer and cyanoacrylate. The use of the TP 407 maintains the lumen of the vein open and prevents it from collapsing. Moreover, it allows a precise approximation of vessel stumps and prevents the risk of glue leakage into the vessel lumen.

Another alternative to the hand-sewn process for venous anastomosis is the coupling device, which gained popularity among the microsurgeons over the last years. The coupler carried out by Synovis Micro Companies Alliance, Inc. presents several advantages over the traditional technique, such as shorter anastomotic time, intima-to-intima contact without interposed foreign material or limited collapse at anastomosis owing to stenting effect of the ring¹¹. Furthermore, Synovis also manufactures the flow coupler, which has an embedded Doppler probe. However, we believe our technique could circumvent a part of the drawbacks of this device and it could avoid some of the complications documented in the literature¹². Head and McKay¹³ reported an increased disposable cost by 284.40 dollars (234.64 euros) for

each venous coupled anastomosis compared with the hand-sewn sutures, while the cost of a 1ml syringe of cyanoacrylate together with the TP407 was estimated at 70 euros. Therefore, performing a sutureless anastomosis could be affordable even for hospitals with limited resources, while using a coupler could become costly, especially when multiple anastomoses are needed in a surgery.

While TP407 and cyanoacrylate use on arteries was already well-documented, there are still reservations against mechanical coupling for arterial repair¹⁴. Thus, the same technique could be performed on both arteries and veins, which decreases the learning curve, as well as total duration of anastomoses. Furthermore, Synovis coupler is intended to be used with veins having an outside diameter between 0.8 and 4.3 mm. Although in our study the mean diameter of the veins was 0.94 mm, the sutureless technique could be easily applied to smaller vessels.

Grewal et al. summarized the literature and reported in their systematic review a mean coupler-assisted anastomotic time of 5.0 minutes¹⁵. Although the mean time of our sutureless anastomoses was 11.9 minutes, this could be explained by the difference of measurement. In our case, the DMA was defined as the time from the section of the external jugular vein to unclamping and not the time actually needed to perform the anastomosis.

Total anastomosis time was almost three times faster in sutureless technique than in hand-sewn technique, although the latter one was performed by a senior surgeon, while the sutureless technique was carried out by a junior surgeon, with no clinical experience in microsurgery. However, sutured or coupler microanastomoses could be also learned by junior surgeons during their residency under standardized conditions¹⁶. Furthermore, we believe that basic microsurgical training should not be forgotten, as a failure of a sutureless anastomosis may require manual suturing.

Skeptics may call in question the safety of using surgical adhesives on vessels, but several studies on a rat model reported no adverse effects to surrounding tissues of the new generation of cyanoacrylates thanks to a rapid polymerization¹⁷⁻¹⁹. Moreover, LeGoo® (developed by Pluromed Inc. USA), was approved by the U.S. FDA for temporary endovascular occlusion of blood vessels below the neck up to 4 mm in diameter²⁰. Thus, this could clarify some questions about the fate of TP407 in the circulatory system.

The present study has some limitations which have to be discussed. Despite the fact that success rates have been similar in both groups immediately after the anastomosis, as well as at day 15 post-procedure, these results have to be confirmed on larger groups in a long-term follow-up. **Feasibility of our technique should be also evaluated when performing end-to-side anastomoses or for veins with different diameters.** Furthermore, a cost-benefit analysis should be made in order to establish the effectiveness of our method. Before application to human, the use of TP407 and cyanoacrylate for venous anastomosis remains to be confirmed in large animal studies.

CONCLUSION:

The short-term safety of this technique was confirmed at the experimental stage. This sutureless method could be reliable for venous microvascular anastomoses and requires lower time than the conventional hand-sewn technique. The short-term safety of this technique was confirmed at the experimental stage. However, additional studies are needed, including in larger animals, in order to consider clinical application in reconstructive surgery.

Disclosure: None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this manuscript.

Conflict of Interest: none

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Legends of figures:

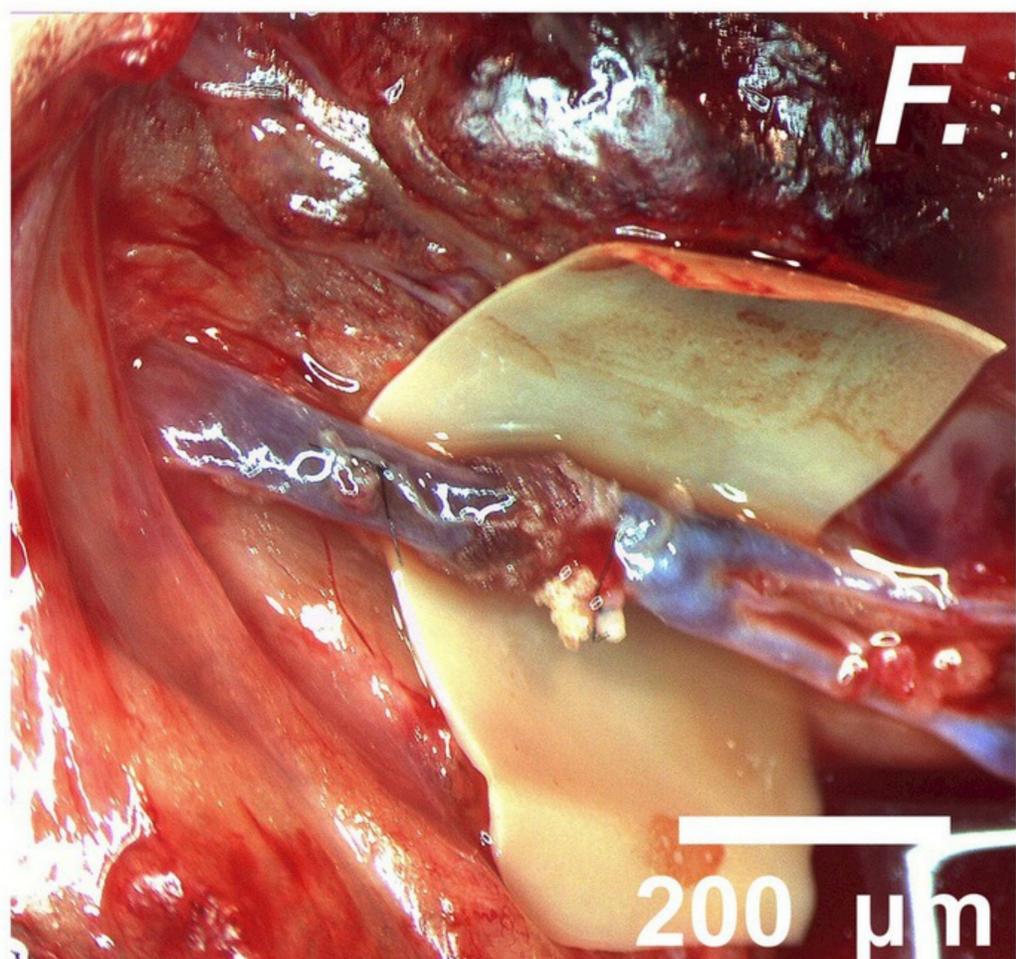
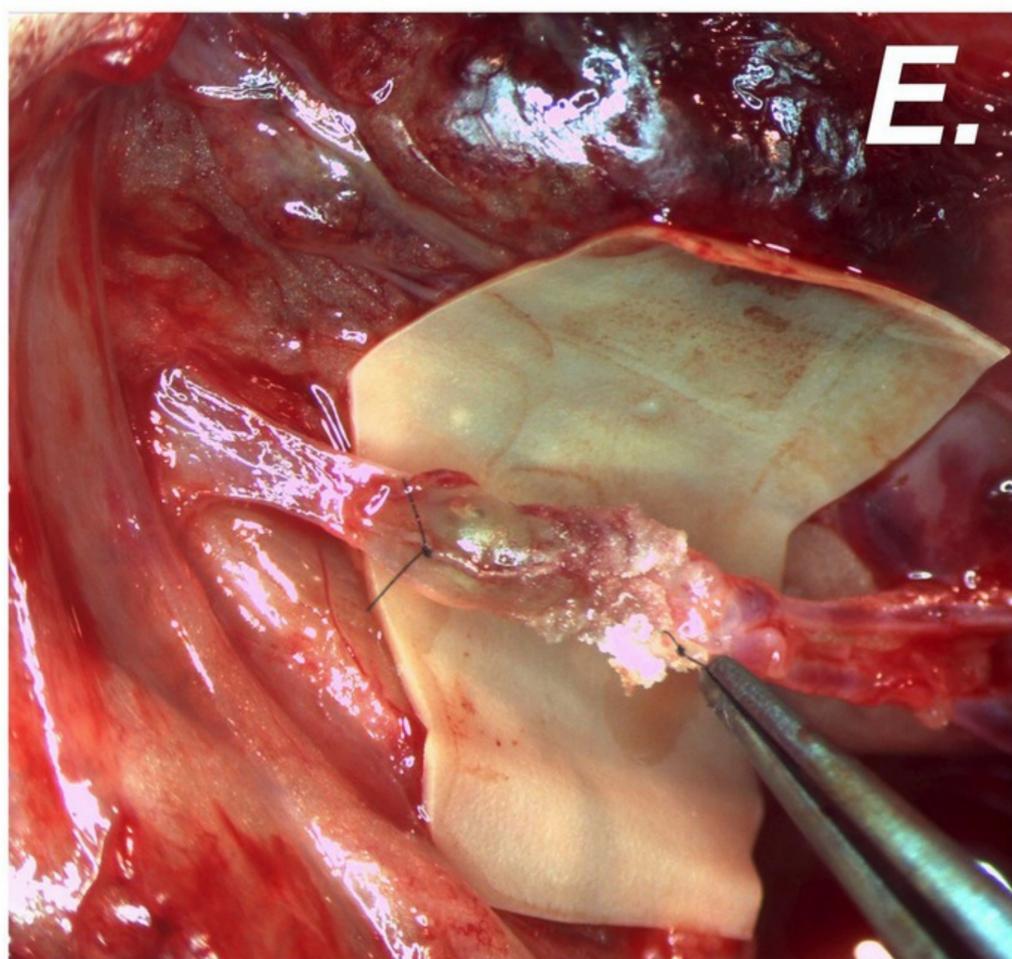
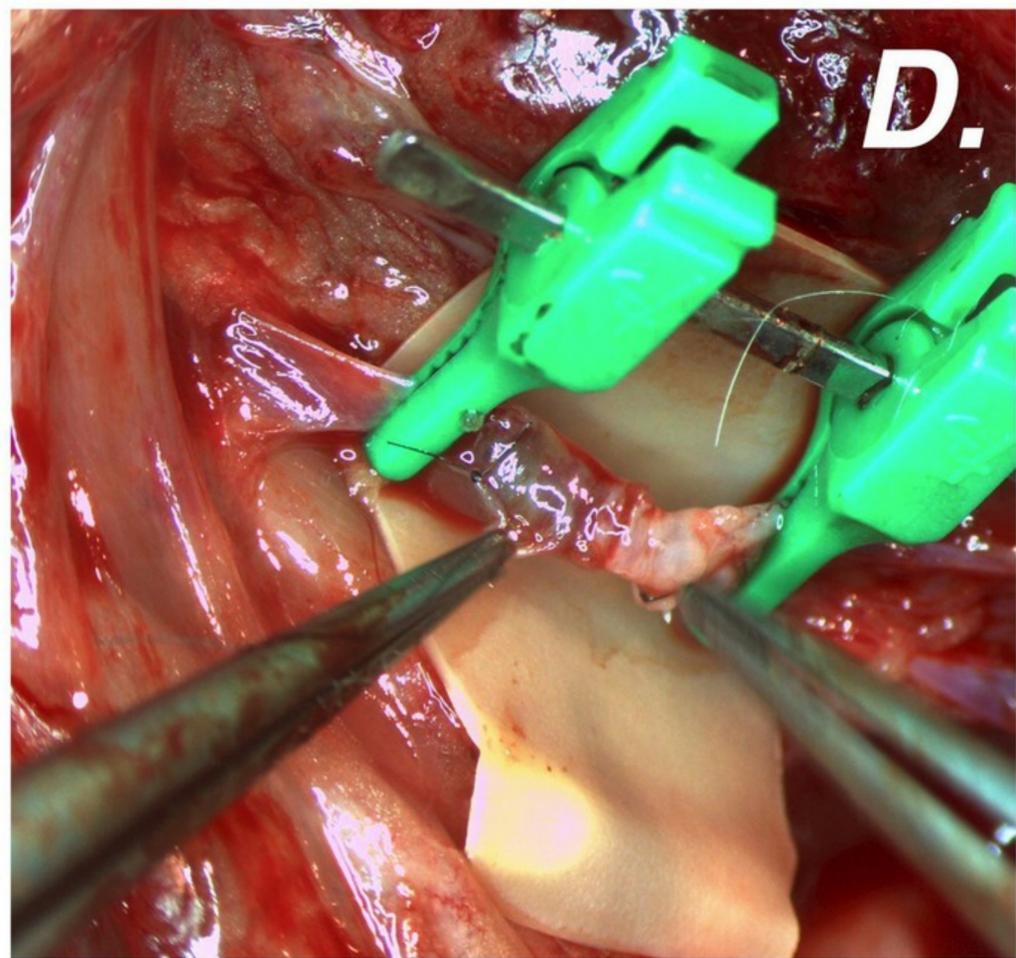
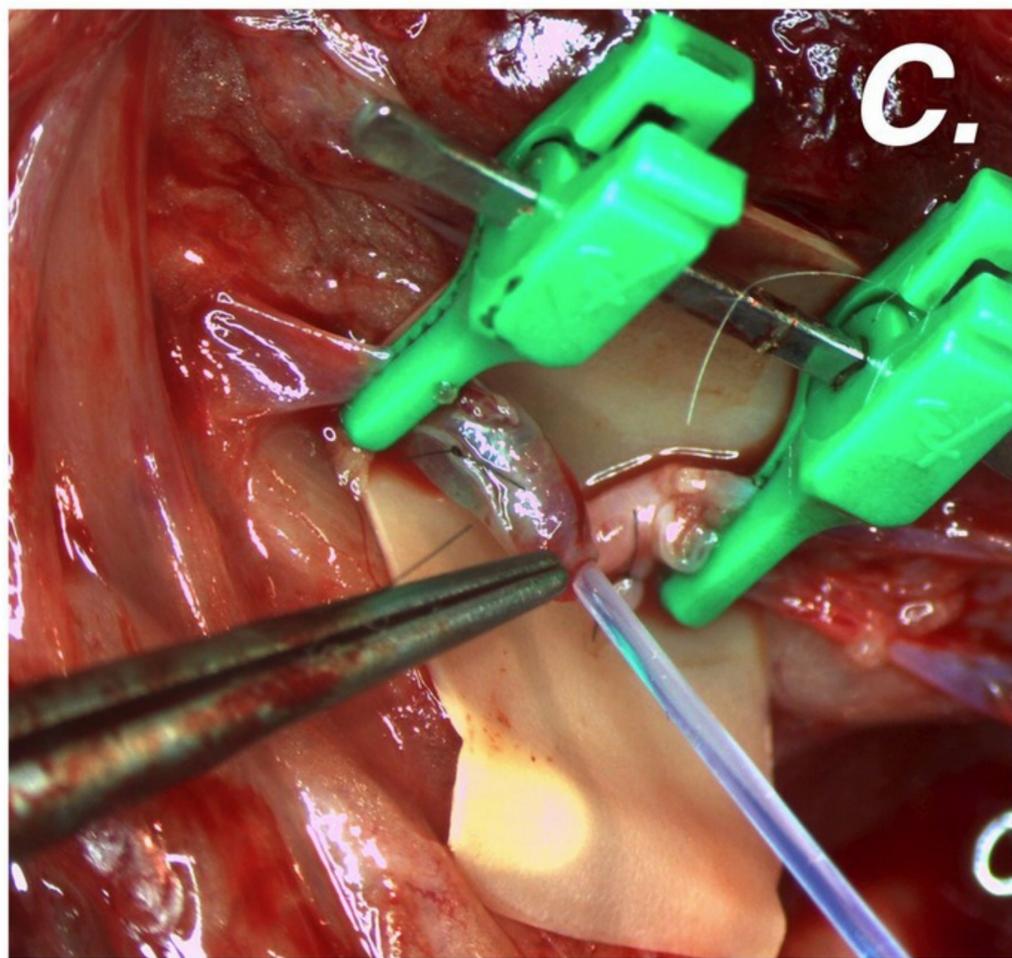
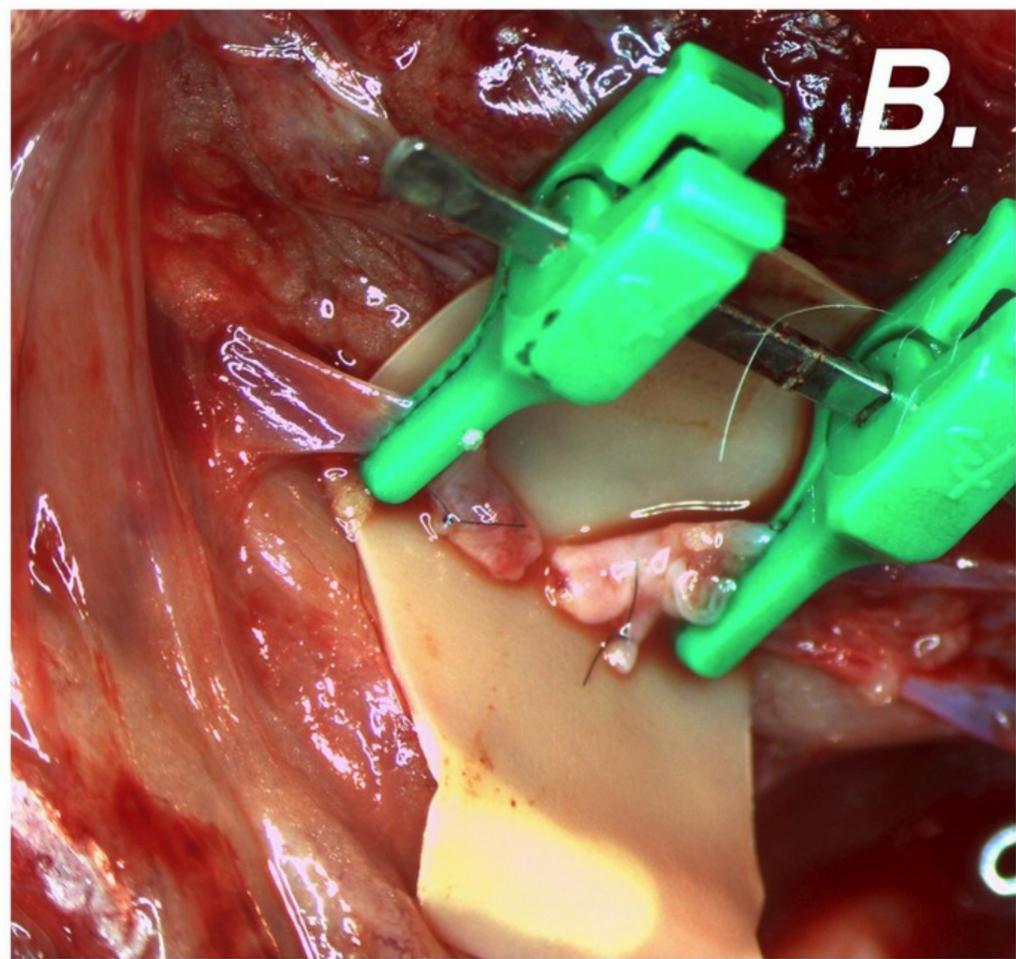
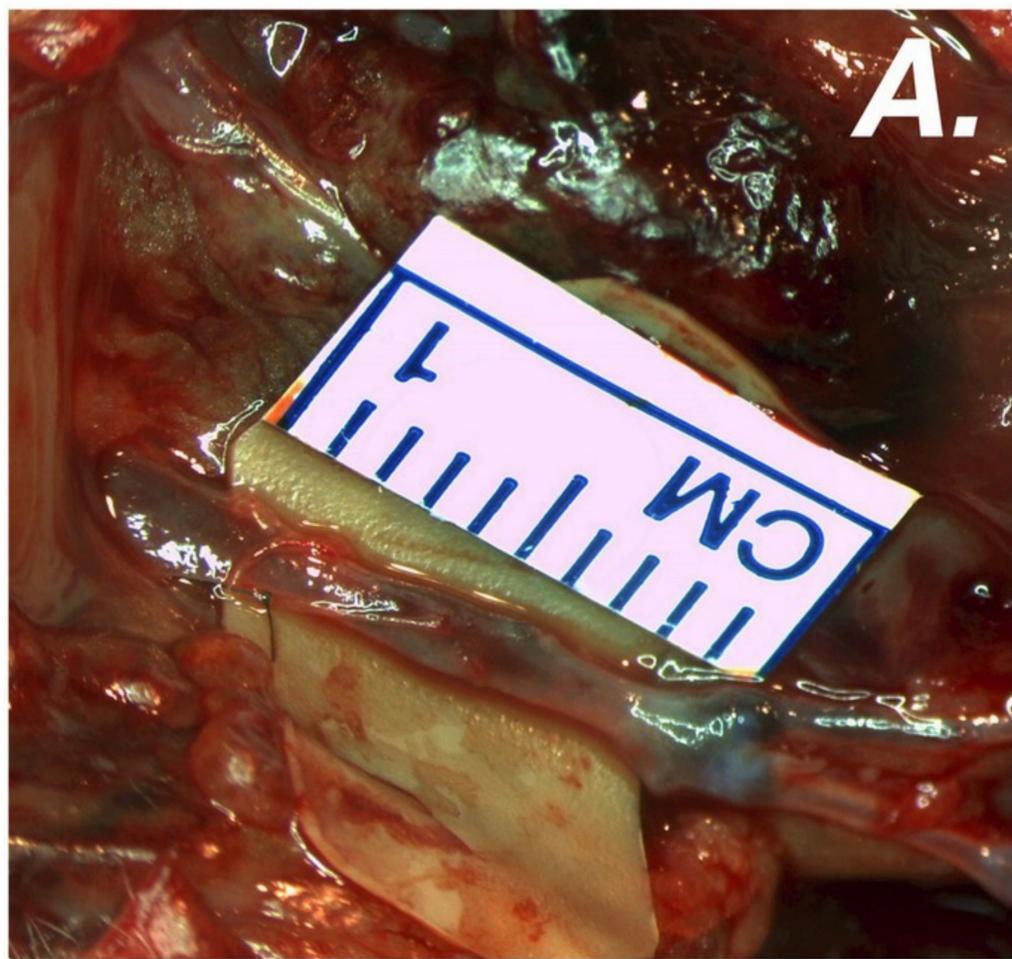
Table 1: Mean and standard deviation values of both groups

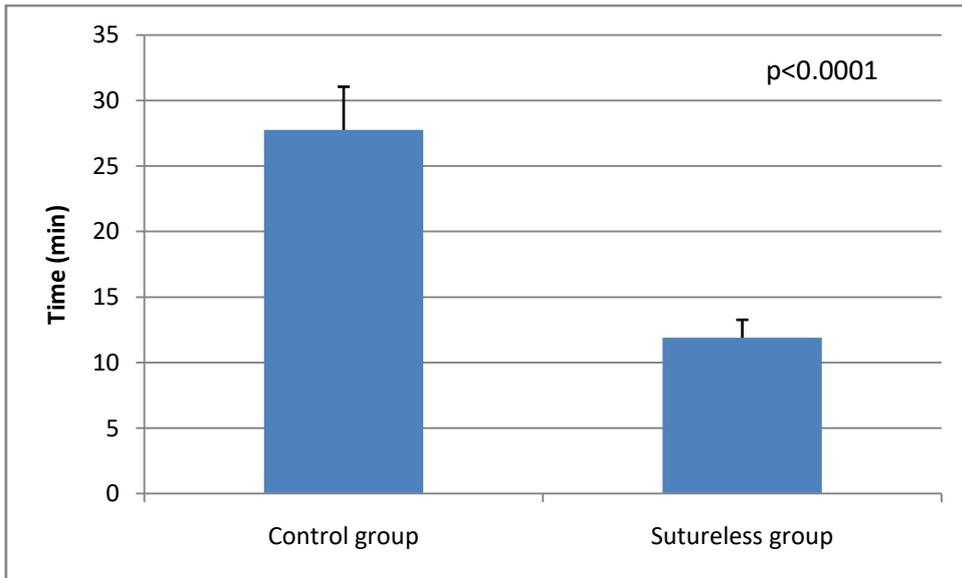
Figure 1: Description of the sutureless method

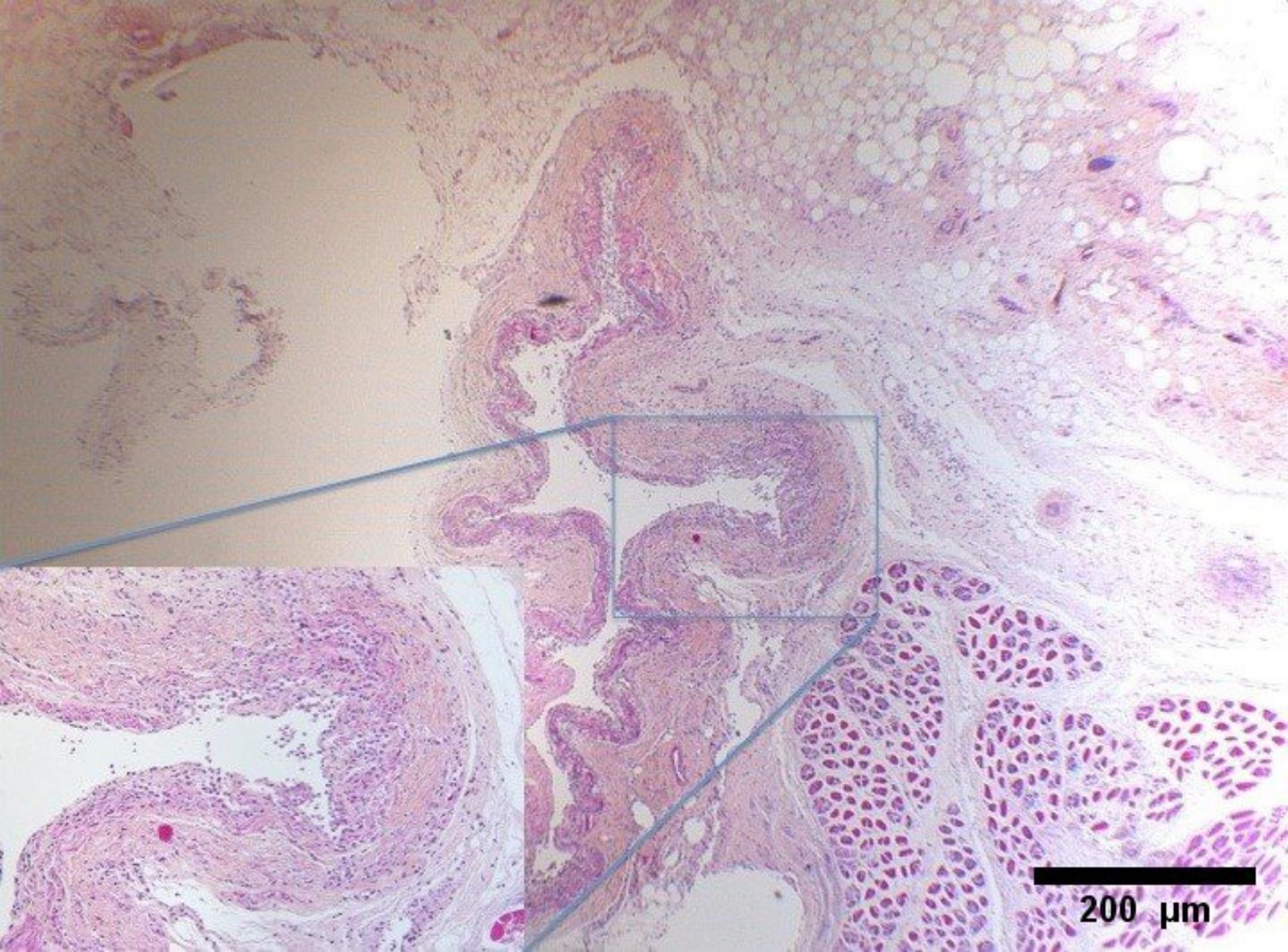
- A. Left external jugular vein after dissection and collaterals ligation.
- B. Transection of the vein. Both ends of the vein are collapsed.
- C. Injection of the thermo-sensitive poloxamer with a 0.7 mm cannula.
- D. Vein stumps are maintained open by the TP 407 at an ambient temperature; approximation could be done without tension.
- E. Aspect after application and polymerization of cyanoacrylate after unclamping.
- F. Aspect after dissolution of the poloxamer spontaneously in contact with blood.

Figure 2: Mean duration of venous anastomoses

Figure 3: Hematoxylin and eosin staining of sutureless anastomosis tissue section at 15 days post-procedure. The numbers of inflammatory cells and multinuclear giant cells were evaluated at anastomosis site (moderate adventitial and media inflammation)







200 μm

Control group	Rat weight D0(g)	Vein diameter(mm)	DMA(min)	Rat weight D15(g)
Mean	287.1	0.95	27.75	285.45
Standard deviation	13.4	0.09	3.31	11.7
Sutureless group				
Mean	287.1	0.9375	11.9	285.45
Standard deviation	13.4	0.1	1.37	11.7

D0: day of the surgery; D1: 15 days post-procedure; DMA: duration of microvascular anastomosis