EVALUATION OF BIOGLUE SURGICAL ADHESIVE
FOR THE PERFORMANCE OF MICROSURGICAL ARTERIAL ANASTOMOSES: IMPACT ON THE FLOW OF VESSELS GREATER THAN 1 MM (RAT AORTA) AND LESS THAN 1 MM (RAT FEMORAL)

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Abstract

Objective. Despite the recent advances and innovations in the field of microsurgery and free flaps, vascular anastomoses are still manual and surgeon dependent with traditional methods. The purpose of this study is to evaluate the effectiveness, in the short and medium-term, of glutaraldehyde with bovine serum albumin (BSA) surgical adhesive in the performance of arterial microanastomoses.

Material and methods. Fourteen femoral end to end anastomoses (Group 1) and 10 aortic anastomoses (Group 2) were performed in 18 Wistar rats. Flux was measured before, immediately after the anastomoses, and 24 hours later, with a transit-time ultrasound to have quantitative data. Anastomoses technique consisted in using minimal stitches to approximate the vessels and applying BioGlue® adhesive to seal the union. The SSPS® package was used for the statistical study.

Results. A median of 2 stitches were necessary in femoral arteries, and 4 stitches in aorta. The median anastomoses time was 16.5 minutes in Group 1 and 32.5 in Group 2. 93% anastomoses of Group 1 and 100% in Group 2, were permeable immediately, and 77% in Group 1 and 100% in Group 2 were permeable after 24 hours, with adequate flux measures. There were no differences in pre and postanastomotic – 24 h aorta-diameter.

Conclusions. The use of glutaraldehyde with BSA is a promising technique for the microsurgery of the future, and it is a fast, easy, and reliable alternative to perform microvascular anastomoses, especially when surgeon needs to avoid repeated trauma in the vessel wall or wants to reduce the material inside the lumen and reduce the risk of thrombosis.

Keywords: microsurgery, vascular anastomoses, sutureless anastomoses, glutaraldehyde, BioGlue, rat model.

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INTRODUCTION

Arterial and venous microsurgery techniques, as well as supramyrosurgery applied to lymphatic vessels, have led to a revolution in reconstructive surgery. Over the last decades there have been great advances in reconstruction with microsurgical flaps, but the way to perform the anastomoses is...
still manual and dependent on the surgeon who must go through a long learning curve [1]. Several mechanical methods have emerged recently, such as the Coupler TM system (Synovis Micro Companies Alliance, Inc. Birmingham, AL) [2, 3] and other sutureless techniques [4], but in general, we can affirm that most centers continue to use the classic microsurgical techniques, especially in arteries, with interrupted stitches or in some cases continuous sutures [5].

The perfect microvascular anastomoses technique must be reliable, fast to reduce the ischemic time, reproducible, and economic. It should avoid trauma to the vessel wall, have adequate short and long-term patency, and be easy to perform and to teach, not requiring a long learning curve. Our objective is to evaluate the efficacy of a substitute to the standard suture, using glutaraldehyde with bovine serum albumin or BSA (BioGlue®, CryoLife International Inc, Kennesaw, Georgia). This compound is being used with success in the repair of large vessels [6–9], but we have found scarce literature [9] regarding its utility in microsurgical techniques. For this reason, we propose a study where we are going to perform interventions on two vessels of different caliber, those measuring between 1mm and 1.5mm, and vessels smaller than 1 mm. For that purpose we have used well-known models in experimental microsurgery such as the rat femoral and aorta arteries [10, 11].

MATERIAL AND METHODS

A total of 18 male Wistar rats were used, laid down following European regulations, with free access to water and food in 12-hour light and dark cycles. Bilateral femoral artery anastomoses was performed in 6 rats, unilateral femoral artery anastomoses was performed in 2 rats, and infrarenal aortic artery anastomoses was performed in 10 rats. All the anastomoses were end-to-end. Two working groups were created to study the efficacy of glutaraldehyde with BSA. Group 1: end-to-end anastomoses of the femoral artery. Group 2: end-to-end anastomoses of the aorta artery. All procedures were performed at the Jesús Usón Minimally Invasive Surgery Center. The study, with registration number 2019209020007902, was approved by the Ethics and Experimentation Committee of the center (File NO: EXP-201911115), according to the regional government welfare guidelines.

A total of 6 different surgeons participated, all of them acquainted with microsurgical techniques. The allocation of animals to be operated on by each surgeon was random.

After weighing the animal, the rat was placed in an anesthesia induction chamber, dispensing oxygen for 5 min (0.5–1 L/min) and a vaporizer was used to administer 5% sevoflurane for anesthetic induction. Subsequently, a mask was placed for inhalation, and a flow rate of 2% sevoflurane was provided for the maintenance of anesthesia.

Eye protection ointment was used. The animal was kept at a temperature of 35.9–37.5 °C, with an O₂ saturation > 95% and a heart rate between 250–450 bpm. Analgesics and anti-inflammatory agents (meloxicam 1 mg/kg/day) and prophylactic antibiotics (enrofloxacin 7.5 mg/kg/day) were given subcutaneously before the procedure and during the 24 hours postoperatively.

The abdominal and inguinal areas of the animal were shaved. The animal was placed under a Zeiss OPMI Pentero 800® microscope, Carl Zeiss AG (Oberkochen, Germany), connected to an external camera that allowed the visualization of the maneuvers that were being performed. The microscope was connected to a photographic camera to obtain images and videos. Subsequently, topical povidone-iodine was applied, followed by 70% ethanol, and the sterile field was set up.

To approach the femoral vessels and aorta, standard techniques were used, performing hemostasis with a bipolar forceps, and using heparinized saline solution (100 U/ml) to clean stagnant blood. Once the vessels were exposed, 2% lidocaine was used as a vasodilator agent, and preoperative flow was measured in ml / sec with AureFlo Unit®, Transonic, Ithaca, USA (Video).

An ABB-1® double micro clamp, S&T AG (Neuhausen, Switzerland) was placed at 3mm distance from the point where a clean cut was made with straight scissors, perpendicular to the vessel direction. The start time of the anastomoses was noted. After washing with heparinized serum, removing the adventitia, and dilating the vessel lumen, we performed a simple stitch with a 10/0 nylon to face both ends. A second stitch opposite the first at 180º was completed to approximate the vessels. The perfect microvascular anastomoses technique must be reliable, fast to reduce the ischemic time, reproducible, and economic. It should avoid trauma to the vessel wall, have adequate short and long-term patency, and be easy to perform and to teach, not requiring a long learning curve.
At that time, an external assistant, maintaining the sterile conditions, prepared and applied a uniform adhesive layer of 0.5mm of BioGlue on the anastomoses (Fig. 1).

It consisted of a double-chamber syringe, and when the plunger was compressed, both substances were mixed in a spiral and after priming the syringe, the BioGlue polymerized quite quickly (Fig. 2). The clamp was then released, and after observing vascular reperfusion, manual patency tests were performed distal to the anastomoses. The end time of the anastomoses was noted, and lidocaine was applied again. To obtain a reliable flow measurement, the probe was kept neutral to the plane of the vessel, neither pulling it nor creating any tension (Fig. 3A). To obtain a good acoustic coupling, saline solution was used. The immediate postoperative flow was recorded by AureFlo® (Fig. 3B). Additionally, comments on the appearance of the anastomoses after the procedure were noted. Once this was done, 4/0 polyglycolic acid (PGA) absorbable braided sutures were used to close the skin, and the animals were placed in their cage for awakening.

After 24 hours, with another general anesthesia, the previous incision was used, and the anastomoses were revised taking flow measurements. Postoperative diameter was measured in aortas, using a microsurgical caliper. In femoral arteries, the diameter was not measured because the adhesive layer did not allow a reliable measurement. The animals were sacrificed with an intracardiac injection of potassium chloride. Samples of the operated vessels were extracted for subsequent pathological study. The vessels were cut longitudinally in such a way that half was stored in glutaraldehyde (for electron microscopy study) and the other half in formaldehyde for study under conventional microscopy.

**Statistic analysis**

The SSPS statastics® package, IBM Corp, Armonk, NY was used for the analytical and descriptive statistical study. For the descriptive analysis, results of medians and interquartile range were obtained. Analytical statistics were performed using
RESULTS

A total of 18 male Wistar rats were operated, with an average weight of 286 g. Bilateral femoral artery anastomoses was performed in 6 rats, unilateral femoral artery anastomoses was performed in 2 subjects, and end-to-end aortic anastomoses was performed in 10 animals. 2 rats from Group 1 died at the end of the procedure, 16 (6 from Group 1 and 10 from Group 2) survived until the next day, and of them, 2 died during anesthetic induction at 24 hours (one from each group).

Immediate post-anastomoses flow measurement, the transmission of the pulsatile impulse, and the patency test confirmed the viability of the anastomoses and the permeability of the vessel.

Descriptive results can be seen in Tables 1 and 2.

**Table 1.** Descriptive data of Group 1 (femoral artery)

<table>
<thead>
<tr>
<th>Animal code (femoral)</th>
<th>Pre-anastomotic flux (ml)</th>
<th>Anast time (min)</th>
<th>Technique (10/0)</th>
<th>Post-anastomotic flux (ml)</th>
<th>24h post-anastomotic flux (ml)</th>
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</thead>
<tbody>
<tr>
<td>Rt20-006(i)</td>
<td>1.2</td>
<td>19</td>
<td>3</td>
<td>1.4</td>
<td>2.2</td>
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<tr>
<td>Rt20-007 d</td>
<td>3.9</td>
<td>33</td>
<td>3</td>
<td>0.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Rt20-007 i</td>
<td>0.7</td>
<td>7</td>
<td>2</td>
<td>0.4</td>
<td>5.0</td>
</tr>
<tr>
<td>Rt20-008</td>
<td>0.9</td>
<td>7</td>
<td>2</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Rt20-008</td>
<td>2.7</td>
<td>32</td>
<td>2</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Rt20-009</td>
<td>5.9</td>
<td>17</td>
<td>2</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Rt20-009</td>
<td>1.5</td>
<td>7</td>
<td>2</td>
<td>1.0</td>
<td>0.3</td>
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<tr>
<td>Rt20-010</td>
<td>0.6</td>
<td>10</td>
<td>2</td>
<td>0.3</td>
<td>–</td>
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<tr>
<td>Rt20-010</td>
<td>2.5</td>
<td>6</td>
<td>2</td>
<td>0.7</td>
<td>–</td>
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<tr>
<td>Rt20-011</td>
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<td>2</td>
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<td>0</td>
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<tr>
<td>Rt20-011</td>
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<td>2</td>
<td>0.3</td>
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<tr>
<td>Rt20-012</td>
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<td>2</td>
<td>0.7</td>
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<tr>
<td>Rt20-013</td>
<td>2.5</td>
<td>19</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rt20-013</td>
<td>1.2</td>
<td>13</td>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean</td>
<td>1.91</td>
<td>17.42</td>
<td>2.35</td>
<td>0.61</td>
<td>0.93</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.49</td>
<td>10.33</td>
<td>0.63</td>
<td>0.39</td>
<td>1.55</td>
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<tr>
<td>Median</td>
<td>1.35</td>
<td>16.5</td>
<td>2.0</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>P25</td>
<td>0.97</td>
<td>7.75</td>
<td>2</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>P75</td>
<td>2.5</td>
<td>20.5</td>
<td>2.75</td>
<td>0.85</td>
<td>1.25</td>
</tr>
</tbody>
</table>

A median of 2.0 (2.0–2.75) points were used in femoral arteries, while in aorta the median was 4.0 (4.0–4.5) points. The total anastomoses time in Group 1 was 16.50 (7.75–20.50) minutes and in Group 2 it was 32.5 (25.5–34.5) minutes.

The preoperative flow in Group 1 (femoral) was 1.35 (0.97–2.50) ml/sec and in Group 2 (aorta) it was 8.05 (6.65–9.05) ml/sec. The immediate postoperative flow was 0.60 (0.30–0.85) ml/sec in Group 1, and 2.70 (1.22–4.35) ml/sec in Group 2.

At 24 hours, the flow increased in the femoral group to 0.70 (0.10–1.25) ml/sec, and in the aortic group to 0.60 (0.55–4.325) ml/sec.

The pre-anastomoses diameter of the rat aortas was 1.15 (1.075–1.425) mm, and the postoperative diameter at 24 hours was 1.20 (1.075–1.25) mm.

**Qualitative description**

It came to our attention from the first moment that the application method is designed for large vessels, and that the content that comes out of the applicator is exaggerated compared to what would be needed for a microvascular anastomoses.

The time it takes for the glutaraldehyde and ASB mixture to set is fast. We had a short learning curve and saw that with large amounts of product, the femoral vessels collapsed. However, this was not the case with the aortas. Once we verified that there was proximal and distal flow in the vessels, as there was an area with a rigid amber appearance, we tried to remove the excess of glue far from the
anastomoses in order to perform a flow record in the area covered by the adhesive.

In some cases, simple clamps were used to prevent the glue from adhering to the bridge of the double clamp, obtaining similar results.

It is essential that the site is dry before applying the product. We saw that when there was a small leak from the anastomoses when placing the product, if we tried to put more adhesive, it was seen that the glue entered in the vessel lumen.

In 2 cases, thrombi were observed in the area where the glue was applied, or glue was observed in the vascular lumen due to an error in the application. In 6 cases, more adhesive had to be added due to anastomoses leakage.

**Comparative study**

Wilcoxon analysis was performed to compare nonparametric data. Statistically significant differences were observed between the pre-anastomoses and immediate post-anastomoses flows in the femoral artery ($p = 0.008$) and aorta ($p = 0.009$). We also observed significant differences when comparing pre-anastomoses and immediate post-anastomoses flows in all the subjects ($p = 0.000$).

In addition, we observed that there were statistically significant differences when comparing pre-anastomoses flows and postoperative flows at 24 hours in all subjects ($p = 0.02$). No differences were found when comparing each group separately (1 group: $p = 0.06$; 2 group: $p = 0.3$).

In the same way, when making comparisons in each group separately, statistical significance was not found in terms of immediate postanastomoses flow and postoperative flow at 24 hours ($p = 0.673$ for the femoral group and $p = 0.327$ in the aortic group).

A Mann-Whitney U test was performed to compare the points that were given to the aorta and the femoral artery, with a median of 4 points (IC range 4–5) in the aorta and 2 points (IC range 2–2, 75) in the femoral, finding statistically significant differences, $p = 0.00$.

When comparing the pre-anastomoses and 24-h postoperative diameters in the aorta with the Wilcoxon test, no statistically significant differences were found ($p = 0.785$).

We were unable to obtain measurements of the femoral vessels since it was difficult to remove all the glue that adhered to the walls, which added millimeters to the diameter of the vessel, thus artificing the results.

Regarding correlations, combinations were made between the number of points required in the intervention and the total time used to perform the anastomoses and we found that a greater number of points led to an increase in the total anastomoses time, with a Spearman correlation coefficient of 0.586 ($p = 0.003$).

**DISCUSSION**

In the present study we have carried out an evaluation of a glutaraldehyde compound with BSA as an alternative to traditional methods to perform microvascular anastomoses in two types of vessels: arteries with a caliber of less than 1 mm (Group 1) and arteries with diameter between 1.0–1.5 mm (Group 2). A widespread model [10–11] of the rat aorta and femoral arteries has been used in order to compare with data from the literature. To avoid bias, 6 surgeons participated in performing the anastomoses, all of them familiarized with microsurgical techniques.

BioGlue is frequently used by cardiac surgeons for great vessel anastomoses [7], it has proven to be very effective in vasosasomisties performed by urologists [12], and it is also useful as a hemostatic agent to prevent seromas [13]. This compound has previously been used in carotid anastomoses in rabbits [9] (with a diameter greater than our vessels), and it has shown to be effective and with a thrombotic occlusion rate similar to the usual suture techniques, but without providing a quantitative measurement of post-anastomoses flow.

However, some authors have seen a severe inflammatory response to BioGlue, even considering glutaraldehyde as a potentially toxic product [14]. Given the small amount needed to perform microvascular anastomoses (a 0.5 mm layer on the vessel, approximately 0.3–0.5 ml of product), which is much less than that applied in surgeries of larger vessels, we consider low the possibility of toxicity in the body.

In microsurgery, other adhesive agents have been used as an alternative to perform microanastomoses with products usually based on fibrin or cyanoacrylate, and with variable results [4, 15–17]. Fibrin polymers are often thrombogenic and must therefore be used with great care at the site of microanastomoses. On the other hand, they may not provide sufficient sealing force at the suture site. There are authors who use fibrin together with vein patches to ensure the success of the intervention [18].

Other authors promote the use of fibrin in microvascular anastomoses in combination with reinforcing stitches. Flap survival is not affected in their experience [19].

In a randomized study in 5 groups, conventional sutures, fibrin gel with accompanying microsutures (similar to our method), the Coupler system, micro-staples, and laser-assisted anastomoses were compared [20]. The authors concluded that the techniques without sutures could be even better than conventional ones for treating veins. Specifically, there are those who consider that pointless anastomoses systems present a level 2b of scientific
Another published method is the use of cyanoacrylate that can be associated with intravascular stents [21]. It has demonstrated safety and reliability in anastomoses, adding rigidity to collapsed veins to avoid suturing the posterior wall of the vessel [22]. However, some studies associate it with a severe long-term inflammatory reaction on histology.

Our work differs from the previous ones in the product used, composed of glutaraldehyde and BSA, with a different methodology. One finding that we want to highlight is the quantitative measurement of flows in the short and medium term, which guarantees the success of the technique.

We carried out measurements in two stages because we wanted to see if this system was effective at the end of the anastomoses and in the short term. As the compound was not designed for microsurgical anastomoses, we needed to check that it worked at 24h and that the stiffness that the adhesive produced in the vessels did not collapse the lumen by external compression.

In our study, 23 of 24 (96%) anastomoses were patent in the immediate postoperative period, observing, immediately after the anastomoses, blood flow volumes higher than the minimum recommended in the literature to predict their long-term patency. G. Shaughness et al. (2017), recommend a minimum flow of 0.3 ml/min for vessels 0.6 to 1.2 mm in diameter to predict adequate vascular patency in the long term [23].

We have observed how the adhesive influences the anastomoses 24 hours after the intervention, also demonstrating that in the aortas the result is persistent and long-lasting (100% patency at 24h), but nevertheless in femoral arteries the patency of the anastomoses to the 24h decreases to 77.7% (7/9).

Although only one of the anastomoses was not patent in the immediate postoperative period, in several cases it was necessary to give an additional stitch or add more adhesive to avoid leaks. In two cases, material that had entered the vascular lumen needed to be removed. It must be considered that while the study was being carried out, we were overcoming the small learning curve that the use of this biological glue entailed.

Being designed for use in large bore vessels, the BioGlue syringe and applicator are oversized that are not commensurate with the precision required in microsurgery. The doses were therefore excessive at first and this effect affected smaller vessels more than larger ones. When the compound set, which happened in a few seconds, the artery was submerged in a kind of amber magma. Although distal flows were present, we were concerned that the block that surrounded the anastomoses might eventually obstruct the flow and therefore it was decided to study the animals 24 hours later. However, we found the opposite, and we believe that the rigidity produced once glutaraldehyde with BSA is applied, could protect the anastomoses from external compression by a nearby hematoma and prevent kinking or twisting of the pedicle.

This technique is simple, fast, and effective, and does not require any special equipment. In addition to completely sealing the anastomoses, if the technique is applied correctly, the foreign material inside the vessel lumen is reduced, since in principle only 2 stitches are needed for femoral arteries and 4 for aortas. Some cases, especially in aortas, required more sutures to obliterate the lumen and avoid the entry of intraluminal glue, hence the variability in the number of stitches.

This system could be very useful applied to lower limb microsurgery, where we frequently find receptor vessels with atheroma plaques or less frequently Mockenhberg’s sclerosis with calcified vessels that have a high risk of tearing with each point and make it difficult to perform manual vascular anastomoses [24]. By requiring fewer stitches than standard techniques and reducing trauma to the vessel wall, the likelihood of a tear is much lower. In addition, ischemia of the vessel wall produced by each point is avoided, achieving a better vascularization of both ends.

Surgeons of various disciplines have used glutaraldehyde and BSA safely over the past decades for their sealing and hemostatic power. However, its role in microsurgical techniques is not certified with endorsement publications.

Even in tests that we have carried out on veins, we have managed to keep the lumen open in small-caliber vessels thanks to the increased consistency of the venous wall.

The patency maneuver or the use of Doppler helps us to identify, but not to quantify, the presence or absence of flow through an anastomoses [24]. C. Krag and S. Holck (1981) observed that almost 50% of the anastomoses that appear patent with the patency test have minimal thrombosis or reduction of the vascular lumen [25]. For this reason, we have used transit-time ultrasound technology, which allows us to quantify blood flow through microsurgical anastomoses [26, 27].

The limitations of our study are the small sample of subjects, the absence of a control group with suture or Coupler anastomoses, the use of BioGlue in venous anastomoses, as well its use in anastomoses of vessels with caliber discrepancies and even end-to-side anastomoses. Another limitation of the present study is the absence of pathological study, which will form part of the second line of research.
CONCLUSIONS

The use of glutaraldehyde with BSA is a promising technique for the microsurgery of the future. If the application form can be standardized and enhanced, it could be an excellent alternative to perform microvascular anastomoses, considering that the results are successful in the aorta and femoral arteries.

REFERENCES


Compared to the traditional methods, this is a fast and easy technique, which does not require a long learning curve. It is reliable in the short and long term, easy to perform and to teach, and avoids repeated trauma to the vessel wall which could be very useful in lower extremity microsurgery in patients with atherosclerosis.

All microsurgeons should be familiar with the application of glutaraldehyde with BSA and resort to this technique if the opportunity arises.

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