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Sutureless microvascular anastomosis with the aid of heparin loaded poloxamer 407

Fırat Özer [a,*], Mustafa Nişancı [a], Çetin Taş [b], Jayakumar Rajadas [c], Doğan Alhan [a], Yalçın Bozkurt [d], Armağan Günal [e], Serdar Demirtaş [f], Selçuk Işık [a]

[a] Gulhane Military Medical Academy, Department of Plastic Surgery, Ankara, Turkey
[b] Gulhane Military Medical Academy, Department of Pharmacy, Ankara, Turkey
[c] Stanford University, Department of Bioengineering, Stanford, CA, USA
[d] Gulhane Military Medical Academy, Department of Radiology, Ankara, Turkey
[e] Gulhane Military Medical Academy, Department of Pathology, Ankara, Turkey
[f] Gulhane Military Medical Academy, Department of Biophysics, Ankara, Turkey

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Summary *Background:* Anastomosis with tissue adhesives is an alternative method for conventional anastomosis. However, this method has several technical challenges. It requires the use of suture to prevent leakage into lumen and precise application onto all surfaces of the anastomosis site. To solve these problems, poloxamer 407 (P 407) was previously used as a stent. In this study, we made heparinized P 407 (h-P 407) as a new formula. We aimed to successfully use h-P 407 as a stent in sutureless anastomosis in a rat abdominal aorta model.
Methods: Sixty Sprague—Dawley rats were used. In the first group, end-to-end anastomoses were performed with suture; in the second and third groups, sutureless anastomoses were performed with 2-octyl cyanoacrylate. As an intraluminal stent, P 407 was used in the second group, and h-P 407 was used in the third group. Anastomosis time was measured. Lumen width, intimal hyperplasia, and foreign body reaction were assessed histologically. Velocity flow rates and vessel diameters were measured radiologically. Burst strength was measured, and the results were statistically evaluated.
Results: Sutureless anastomosis was more rapid than conventional anastomosis. Lumen width was narrower in the suture group. İnflammation and foreign body reaction were more severe in the suture group. There was no radiologic and biomechanical difference between the groups. We found that intimal hyperplasia was less in h-P 407 than in P 407.

* Corresponding author. Department of Plastic, Reconstructive and Aesthetic Surgery, Gülhane Military Medical Academy, Turkey. Fax: +90 4423172264.
E-mail address: ozerfirat@yahoo.com (F. Özer).

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Conclusion: h-P407 can be successfully used as an intraluminal stent for sutureless microvascular anastomosis with tissue adhesives.

Introduction

Vascular anastomosis is the most critical step in all microsurgeries. The conventional hand-sewn method remains the most preferred technique. However, prolonged surgery, technical difficulties, intimal hyperplasia, and foreign body reaction may all render this technique challenging with major disadvantages.[1,2] To overcome the technical difficulties and possible suture-related complications, many alternative techniques have been defined. However, no superior method was found when compared with the conventional hand-sewn suture method.[3–6] Anastomosis using 2-octyl cyanoacrylate as a tissue adhesive is one such alternative method. This method required further refinement for its possible use in routine surgical practice because of adhesives leakage into the lumen.[1]

Edward et al. used poloxamer 407 (P 407) gel as a stent in vessel ends and performed sutureless anastomosis using 2-octyl cyanoacrylate.[7] Chemically, poloxamers are a triblock of nanoparticles namely two polyethylene oxide and one polypropylene oxide chains. Thermoreversibility is a noteworthy property of P 407, i.e., P 407 can be in gel phase at above the transition temperature and it can turn back to liquid phase at the body temperature. When it is in the gel form, P 407 has been shown to have adequate elastic modulus to maintain an open vessel lumen. Moreover, in pharmaceutical technology, poloxamer gels have been used as delivery vehicles for various agents such as chemotherapeutics, vaccines, and antiviral drugs.[8–10]

In the present study, we formulized, produced, and used heparinized P 407 (h-P 407) solution as a stent for sutureless anastomosis using 2-octyl cyanoacrylate.

Material and methods

Preparation of formulation

The poloxamer was prepared as described by Edward et al.[7] Briefly, 1.65 g P 407 (BSAF, Parsipanny, NJ, USA) and 0.025 g BSA (Millipore, Bedford, MA, USA) were mixed, and phosphate buffered saline was added to yield a 10 ml of solution. In total, 5000 units (IU) of heparin (St Louis, MO, USA) was added into this basic formulation, which was used for the third group. Before using this new solution, we tested whether heparin mixed with the liquid phase of the poloxamer in a diffuse manner. The formulation that we finally achieved (16.5% P 407 and 0.25% BSA) provided adequate elastic modulus at 39–40 °C to maintain an open vessel lumen, and it could safely revert to its liquid phase at the physiologic body temperature in in vitro studies.

Animals

A rat abdominal aorta model was preferred for our experimental study.[11] Sixty Sprague–Dawley rats, weighing 300–350 g each, were used after they were divided into three groups: hand-sewn suture group, pure P 407 group, and h-P 407 group. Each group consisted of 20 rats. The study was performed according to the procedures approved by the Animal Research Committee of Gülhane Military Medical Academy. All the animals used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the ethics council.

Surgical procedure

All surgical procedures were performed under general anesthesia. General anesthesia was induced with intramuscular ketamine 10% (90 mg/kg) and xylasine 2% (10 mg/kg). Abdominal aorta dissections and anastomoses were performed under 20× surgical magnification (Carl Zeiss, OPMI Pentero, Germany).

Median laparotomy incision was performed under general anesthesia. Intestines were lateralized and covered with warm sterile gauze dressing. The abdominal aorta segment (so-called infra renal aorta, which is approximately 13 mm in length) between the renal branches and iliaca bifurcation was dissected. The abdominal aorta branches into two iliolumbar branches in the middle of this segment, and these two branches were ligated with sutures instead of coagulation to prevent thermal damage to the anastomosis site. Consequently, the ligated iliolumbar vessels, which were located near the anastomosis site, were not related with the anastomosis (Figure 1). After this step, the infrarenal aorta was surgically exposed, and its adventitia was denuded off. End-to-end anastomoses were performed at this level of the aorta. The lumens were irrigated with serum saline before anastomosis in all groups. No vasodilatory agent was used in this step of the procedure. An Acland-type approximator was used for anastomoses.

In the first group (suture group), hand-sewn anastomoses were performed with 10–12 interrupted 10/0 nylon sutures (Doğsan®, Turkey). In the second group, sutureless anastomoses were performed using 2-octyl cyanoacrylate (Glubran®, Italy), and in this group, P 407 was used as a stent. In the third group, sutureless anastomoses were performed using 2-octyl cyanoacrylate (Glubran®), and h-P 407 was used as a stent.

In the second group, 0.3 ml P 407 gel was injected into the vessel lumens. In the third group, 0.3 ml h-P 407 (containing 150 IU heparin[12]) was injected into the vessel

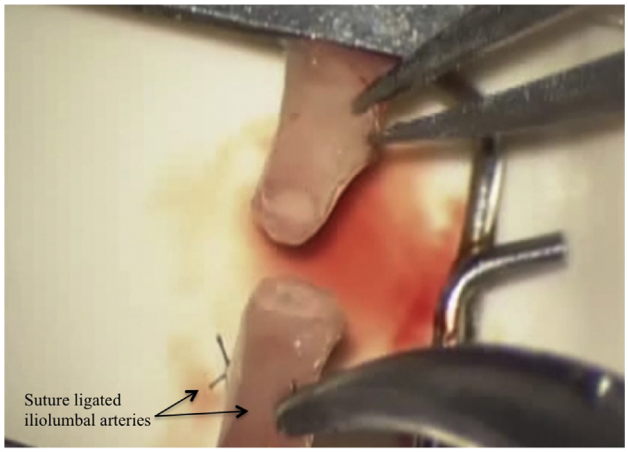

Figure 1 Poloxamer gel, which is injected at 40 °C (above transition temperature), maintains open vessel ends during the 2-octyl cyanoacrylate application. Iliolumbar vessels that were ligated with sutures are located near the anastomosis site. Consequently, sutures, which are seen in the figure, are not related with anastomosis.

lumens. In these poloxamer groups, both P 407 formulations (pure and heparinized) were heated above the transition temperature (39—40 °C) before their infusion into the divided vessel ends to maintain an open lumen (Figure 1). To achieve maximum elastic modulus during the procedure, radiant halogen heat source was used to maintain the temperature of the surgical field just above the phase-transition temperature. The temperature was monitorized and was held below the 43 °C threshold of thermal damage.[13] After the exact approximation of the gel-filled lumens of the vessel ends, 2-octyl cyanoacyrlate was applied in a circumferential manner (Figure 2) to complete the anastomosis. Before releasing microvascular clamps, we approximately waited for 5 min for the polymerization of 2-octyl cyanoacyrlate.

At the end of the surgery, patencies were confirmed by milking test. No intraoperative or postoperative complication was encountered in either group. All rats survived without surgery-related complications (Video 1) .

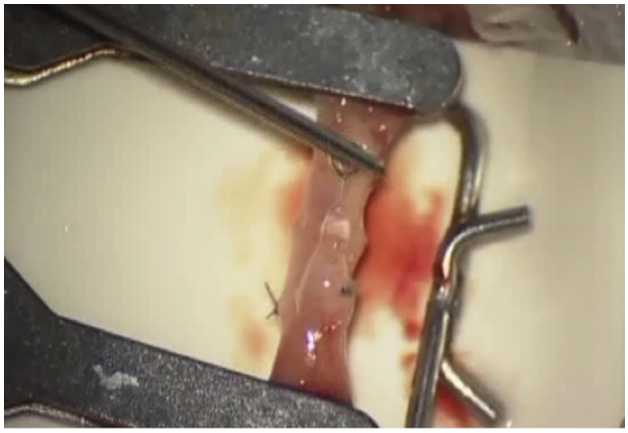

Figure 2 2-Octyl cyanoacrylate was applied in a circumferential manner.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.bjps.2016.10.012.

Evaluation

In each group, data pertaining to the measurements of anastomosis time (intraoperative), radiologic imaging (in the sixth week), histological investigations (in the first and sixth weeks), and biomechanical tests (in the sixth week) were obtained and recorded for comparison.

Anastomosis time was described as the time elapsed from arteriotomy until the removal of vessel clamps following anastomosis and measured intraoperatively ($n = 20$).

In the first week, 10 rats were sacrificed in each group for histological investigations. Vessel segments of 18 mm that included the anastomosis sites were sampled and fixed in a 10% formalin solution for 24 h. The specimens were embedded in paraffin blocks, and then 4-micron thickness sections were obtained and stained with hematoxylin and eosin. Histologically, lumen width and vessel wall thickness were measured (DP Manager-Olympus BXSI, Olympus Co, Japan), and the number of inflammatory cells and multinuclear giant cells was counted at the anastomosis site in a high-power field (400×) (Figure 3).

In the sixth week, the abdominal aortas between diaphragmatic hiatus and iliac bifurcation were assessed using Doppler ultrasound (14 mHz linear probe; LOGIQ Ultrasound, General Electric). This examination enabled us to obtain intravital measurements of the vessel diameters and the blood flow rate ($n = 10$). CT angiogram images were obtained using TOSHIBA Aquilion spiral CT with a 320 detector. The images were obtained using 1-mm vessel slice thickness and 0.5 intersection gaps. These angiograms were utilized to assess the vessel diameters more precisely ($n = 10$).

In the sixth week, after radiologic imaging, histological evaluation was performed ($n = 5$). The same technique was used and same parameters were evaluated as those in the first-week histological investigation.

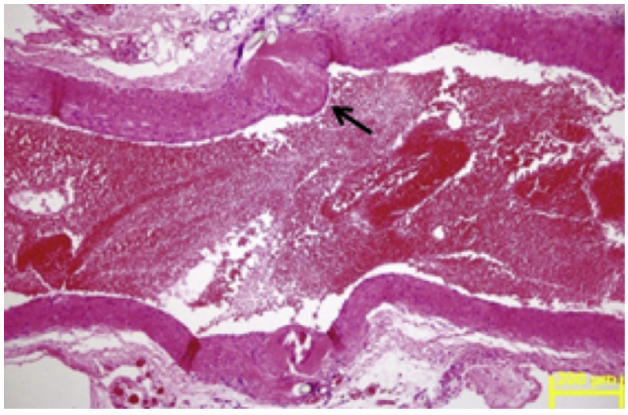

Figure 3 H&E staining of hand-sewn anastomosis tissue section 6 weeks after operation. Arrow shows anastomosis site. Lumen width, vessel wall thickness, and the number of inflammatory cells and multinuclear giant cells were evaluated at the anastomosis site in high-power field (400×).

Burst strengths of the anastomoses were measured using a custom-designed system in the sixth week (n = 5). Aorta segments of 18 mm were used. One end of the vessel was fixed, and the other end was pulled at 0.05 cm/s to the opposite direction using a custom-made device. The values were measured and recorded at the time when the anastomosis sites were broken down.

Statistical analysis

Statistical analysis was performed with SPSS version 15.0 statistical package program. Descriptive statistics were presented as mean and standard deviation. One-way ANOVA and post hoc Tukey test were used to compare groups. A *p* value of <0.05 was accepted as statistically significant.

Results

Anastomosis time

The recorded times elapsed for anastomoses revealed that traditional hand-sewn anastomosis was much more time-consuming, with a mean anastomosis time of 35.5 ± 1.8 min, compared to the sutureless technique that had a mean anastomosis time of 8 ± 0.8 min ($p < 0.001$).

Imaging techniques

Doppler ultrasound examinations revealed no observable differences in vessel diameters (1.1 mm in all groups, $p = 1.00$) (Figure 4) and only verified the patency of anastomosis sites with similar volumetric flow rates (14.3 ± 0.5 cm/min in the suture group, 14.6 ± 0.5 cm/min in the P 407 group, and 14.5 ± 0.6 cm/min in the h-P 407 group; $p = 0.568$). In addition, the patencies were also confirmed with CT angiogram studies without any statistically significant difference between the groups (1.1 mm in all groups; $p = 1.00$) (Figure 5).

Histological evaluations

In both early and late histological investigations, lumen widths were found to be narrower in the suture group than in the two sutureless groups in the first ($p < 0.001$) and sixth weeks ($p = 0.001$) (Table 1), whereas there was no statistically significant difference between P 407 and h-P 407 groups for lumen width ($p > 0.05$).

Histological measurements of the vessel wall thickness revealed that the vessel walls were thicker in the suture group both in the first ($p < 0.001$) and sixth weeks ($p < 0.001$) (Table 1). In addition, the measurements obtained in the first week disclosed that the vessel walls were significantly thinner in the h-P 407 group than the in the P 407 group ($p < 0.001$) (Figure 6). In the sixth week, there was no statistical difference between P 407 and h-P 407 groups for vessel wall thickness ($p = 1.00$).

More number of inflammatory cells were counted in the suture group than in the sutureless groups in the first week ($p < 0.001$), and more number of inflammatory cells were counted in the P 407 group than in the h-P 407group ($p < 0.001$). No statistically significant difference was observed between the groups for inflammatory cell counts obtained in the sixth week ($p > 0.05$) (Figure 7). On the contrary, the number of giant cells was significantly higher in the suture group than in the sutureless groups in the sixth week ($p < 0.001$), whereas there was no statistically significant difference between the giant cell counts of all groups in the first week ($p = 1.00$).

Biomechanical tests

The mean burst strength force was 40 g force in all groups ($p = 1.00$). The only statistically significant difference in burst strength force was obtained when a native vessel (200 g force) was compared with a vessel from the study groups ($p = 0.001$).

Discussion

When bioadhesives were introduced as novel products that can facilitate many surgical procedures, researchers were intrigued by the idea of joining vessels using an available tissue adhesive in a sutureless manner.[14,15] Indeed, the use of tissue adhesives instead of sutures may be a fascinating technique for performing anastomosis that can circumvent the major disadvantages associated with the classical suture technique including more time consumption and challenging surgical labor, vessel wall trauma, and foreign body reaction against suture material.

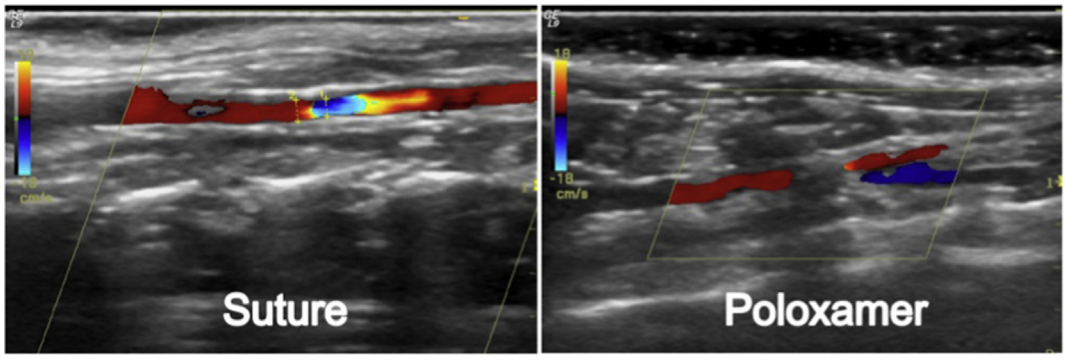

Figure 4 Anastomoses sites are shown with Doppler ultrasound.

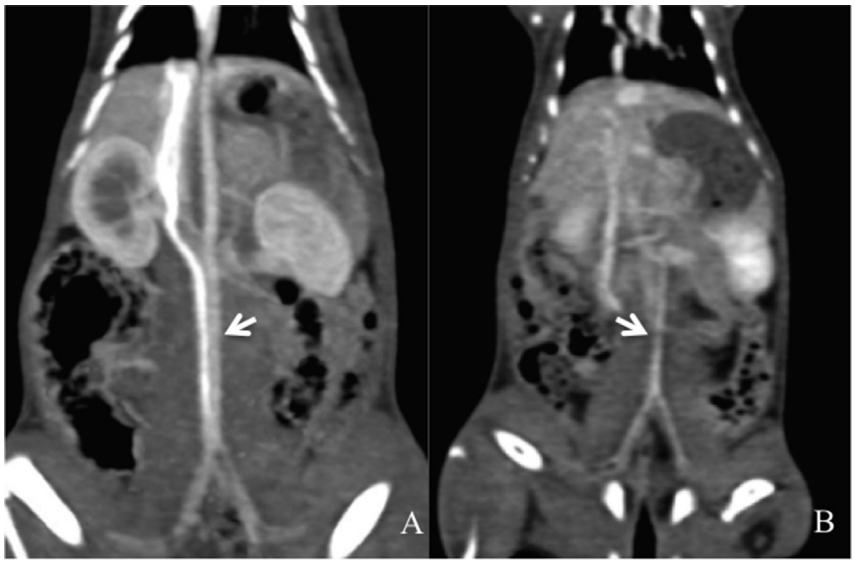

Figure 5 CT angiogram images of the infrarenal aorta. Anastomoses sites are marked with arrows. (A) Anastomosis site in the suture group. (B) Anastomosis site in the sutureless groups.

Table 1 Vessel lumen is narrower in the suture group than in the sutureless groups in the first and sixth weeks. Vessel wall is thicker in the poloxamer group than in the poloxamer groups in the first and sixth weeks. In addition, vessel wall is thinner in the h-P 407 group than in the P 407 group.

	Lumen width (μm) (mean ± SD)		Vessel wall thickness (μm) (mean ± SD)	
	1st week	6th week	1st week	6th week
Suture	465.0 ± 4.0	570.0 ± 1.414	140.0 ± 2.748	120.0 ± 2.000
P 407	474.0 ± 6.1[a]	575.0 ± 1.581[a]	122.0 ± 1.247[a]	90.0 ± 1.581[a]
h-P 407	472.2 ± 1.7[a]	574.0 ± 2.645[a]	95.0 ± 1.563[a,b]	90.0 ± 1.581[a]
P	<0.001	0.001	<0.001	<0.001

[a] p value shows difference between poloxamer groups with suture group.
[b] p value shows difference between h-P 407 group with P-407 group.

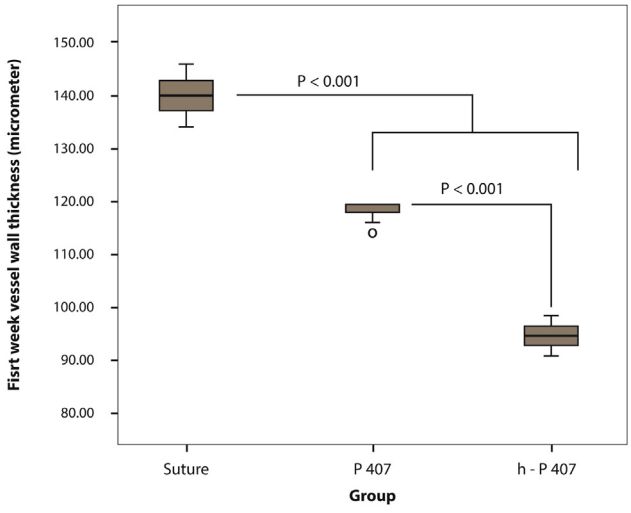

Figure 6 Vessel wall was thicker in the suture group than in the sutureless P 407 and h-P 407 groups. In addition, vessel walls were thinner in the h-P 407 group than in the P 407 group.

However, the requirement for precise alignment of the vessel ends while maintaining open lumens and the almost inevitable leakage of the adhesive into the vessels appear to be the major drawbacks of any technique in which tissue adhesives may be used instead of sutures.[16] Although sutures can be anchored to ensure a precise end-to-end alignment with open lumens, this will certainly invalidate the advantages attributed to using tissue adhesives instead of sutures. Therefore, ongoing researches have recently focused on developing a better technique that can overcome the limitations of using tissue adhesives for anastomosis.

To achieve a patent anastomosis by joining vessel ends with tissue adhesives, using a transient intravascular stent is advisable in terms of not only maintaining an open lumen during the procedure but also preventing adhesive leakage into the vessel. Accordingly, the search for a proper intraluminal stent has become a new subject of interest for researchers. Recently, Edward et al.[7] studied the P 407 gel in detail for its potential use in sutureless anastomosis and then successfully performed the first completely sutureless microvascular anastomosis in a rat abdominal aorta model

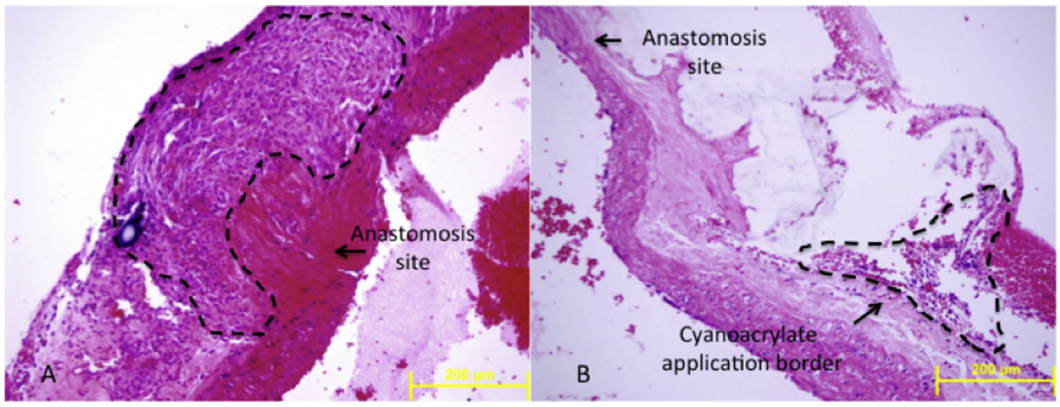

Figure 7 Inflammatory cells were observed at the anastomoses sites. (A) Inflammatory cells located at the anastomosis site in the suture group (B). Inflammatory cells accumulated around the 2-octyl cyanoacrylate application site than at the anastomosis site in the sutureless groups.

using this gel as an intraluminal stent. In addition, Ying-Zheng et al.[17] designed a novel P 407—low-molecular-weight heparin solution and successfully used this new hydrogel for sutureless anastomosis. They reported no undesirable changes in the thermoreversibility of P 407.

From the aforementioned studies,[18—21] we added a therapeutic dose of heparin into the P 407-formulation to obtain an antithrombotic-loaded intravascular gel stent that can be used in sutureless microvascular anastomosis. We expected the h-P 407 to release heparin locally while it dissolves at the body temperature. In the h-P 407 group, we injected 0.3 ml of poloxamer gel containing 150 U of heparin into vessel ends and noticed no heparin-related hemorrhagic complication.[12,22]

Total anastomosis time was four times more rapid in the sutureless technique than in the hand-sewn technique. No significant difference was observed between the P 407 and h-P 407 groups. We did not find any difference among the groups for imaging evaluations and burst strength. Unlike Edward et al.,[7] we found that the burst strength was five-fold weaker compared with that of the native untouched vessel.

In histological evaluations, we found no significant differences in terms of lumen width, inflammation, and foreign body reaction in the first and sixth weeks. The most striking result that we achieved was the statistically significant difference between the P 407 and h-P 407 groups in terms of vessel wall thickness in the first week. We inferred that this was due to thinner pseudointima formation in the h-P 407 group. However, it could have been better observed with labeled heparin, showing its releasing from the gel. Therefore, a disadvantage of this study is that we could not estimate whether the effect of heparin is topical or systemic. We believe that residual heparin is likely to be at the anastomosis site, but this requires to be verified with further investigation.

However, before applying this method to humans, some questions regarding the fate of poloxamer in distal circulation and end organs should be clarified. Consequently, further studies are necessitated. In our study, we performed anastomosis on same size vessels. Therefore, this technique is more suitable for vessels of same size. This method can be applied to both arteries and veins.

In conclusion, P 407 can be used as a reliable intraluminal stent to facilitate sutureless microvascular anastomosis with tissue adhesives, and when it is added with heparin, early probable thrombotic complications due to thick pseudointima formation may be reduced.

Funding

None.

Conflicts of interest

None declared.

Ethical approval

Not required.

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