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ORIGINAL ARTICLE

Supermicrosurgery and hyaluronic acid: Experimental feasability study of a new method

Supermicrochirurgie et acide hyaluronique : étude expérimentale de faisabilité d'une nouvelle méthode

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Hyaluronic acid;
Microsurgery;
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Summary
Introduction. — As a technique of anastomosis for vessels of less than 0.8 mm in diameter, supermicrosurgery has aroused renewed interest on account of its potential clinical applications. The technical difficulty of surgery at such a small scale necessitates exploration of new methods likely to render the anastomoses accessible and reliable. The aim of this article is to present the results of an experimental study on the feasibility of anastomoses (arterial diameter $\leq$ 0.5 mm), assisted by the injection of hyaluronic acid (HA).
Materials and methods. — Ten end-to-end arterial anastomoses of the inferior epigastric artery (diameter $\leq$ 0.5 mm) were performed in 5 rats. An injection of HA had previously been carried out in the vessel lumen and the sutures were made with 12—0 nylon. Immediate and 3-day permeability were controlled and anastomosis times were measured.
Results. — Average diameter of the arteries was 0.42 mm (range 0.29—0.48 mm). Mean anastomosis duration was 19.5 min (range 15—23 min). The average number of stitches was 6. Immediate patency was 100% with a success rate of 80% at 3 days.
Conclusions. — The properties of HA seem to effectively facilitate anastomoses of arteries with a diameter $\leq$ 0.5 mm. HA provides comfort and promotes safety in performance of exceedingly

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small-scale surgery. While the results appear promising, but further studies are needed in order to determine the potential toxicity of this method on tissues.

MOTS CLÉS
Acide hyaluronique ;
Microchirurgie ;
Supermicrochirurgie

Résumé
Introduction. — Les anastomoses dites supermicrochirurgicales concernent les vaisseaux de diamètre inférieur à 0,8 mm et rencontrent un regain d'intérêt devant les applications cliniques qui peuvent en découler. La difficulté technique à cette échelle nous incite à rechercher des méthodes qui rendraient ces anastomoses accessibles et fiables. Nous présentons les résultats d'une étude expérimentale de faisabilité d'anastomoses d'artères de diamètre $\leq$ 0,5 mm assistées par l'injection d'acide hyaluronique (AH).
Matériels et méthode. — Dix anastomoses termino-terminales de l'artère épigastrique inférieure (diamètre $\leq$0,5 mm) ont été réalisées chez 5 rats. Une injection d'AH a été effectuée préalablement dans la lumière des vaisseaux, puis les sutures étaient réalisées au fil 12—0. La perméabilité immédiate et à 3 jours a été contrôlée et les temps d'anastomoses ont été mesurés.
Résultats. — Le diamètre moyen des artères était de 0,42 mm (min. 0,29—max. 0,48). Le temps moyen pour la réalisation d'une anastomose était de 19,5 min (min. 15—max. 23). Le nombre moyen de points de suture était de 6. La perméabilité immédiate était de 100 % avec un taux de succès de l'anastomose de 80 % à 3 jours.
Conclusions. — Les propriétés de l'AH semblent être un atout pour faciliter les anastomoses d'artères de diamètre $\leq$ à 0,5 mm. L'AH offre un confort et une sécurité non négligeable lors de la réalisation de gestes à une si faible échelle. Les résultats semblent prometteurs mais nous encouragent à des études plus poussées sur la toxicité éventuelle de cette méthode sur les tissus.

Introduction

Supermicrosurgery is a technique for anastomosis of vessels of less than 0.8 mm in diameter that has aroused renewed interest on account of its potential clinical applications [1—3]. Perspectives for new therapeutic strategies in a wide variety of areas [3—7] (hand surgery, lymphedema treatment, tissue transfers from perforator to perforator, etc.) have enthused numerous plastic surgeons, and the recently published Barcelona consensus conference [8] met their expectations in terms of definition while recognizing the currently limited dissemination of these methods. Indeed, the technical difficulties encountered on such a small operative scale restrict today's clinical applications of supermicrosurgery.

While it is likely that these techniques are to be incorporated in microsurgery teaching programs that will increase their notoriety, it remains the case that new methods need to be explored in view of rendering the anastomoses reliable and reproducible. In this respect, a number of approaches have already been put forward [9—12], each of them presenting its particular advantages and drawbacks, but supermicrosurgery has yet to be democratized. That is why we are presenting the results of an experimental feasibility study of a new method designed to support supermicrosurgical anastomoses through injection of hyaluronic acid (HA). Since this study was conducted with a model comparable to our published work on the temporary intravascular stent technique (IVaS) in supermicrosurgery [13], comparison with the new technique we are proposing in this paper should be achievable.

Material and methods

Our working model was an in situ inguinal flap placed on the inferior epigastric pedicle in the rat. After lifting the vascular pedicle island flap, the inferior epigastric artery was sectioned and then reconnected on site by anastomosis.

Animals

The work was carried out in 5 rats of the Sprague-Dawley strain. Anesthesia was achieved with an intraperitoneal solution consisting of diazepam at 5 mg/kg and ketamine at 100 mg/kg, with an additional dose when overall manipulation time exceeded 60 min. The surgical area was shaved and then disinfected with a chlorhexidine gluconate solution.

In each rat, two anastomoses of the inferior epigastric artery were subsequently carried out, with the operation systematically starting on the left side. The same surgeon performed both anastomoses on the same animal.

Surgical technique

The operative schema and the flap lifting technique (Fig. 1a) were the same as those used in the method described by Gilbert et al. [14]. As was already the case in our previous work [13], the specificity of our surgical procedure lay in our preparation of the epigastric vessels.

In the classic in situ inguinal flap technique, the epigastric pedicle of the rat is not dissected and the anastomoses are carried out at the level of the femoral artery.

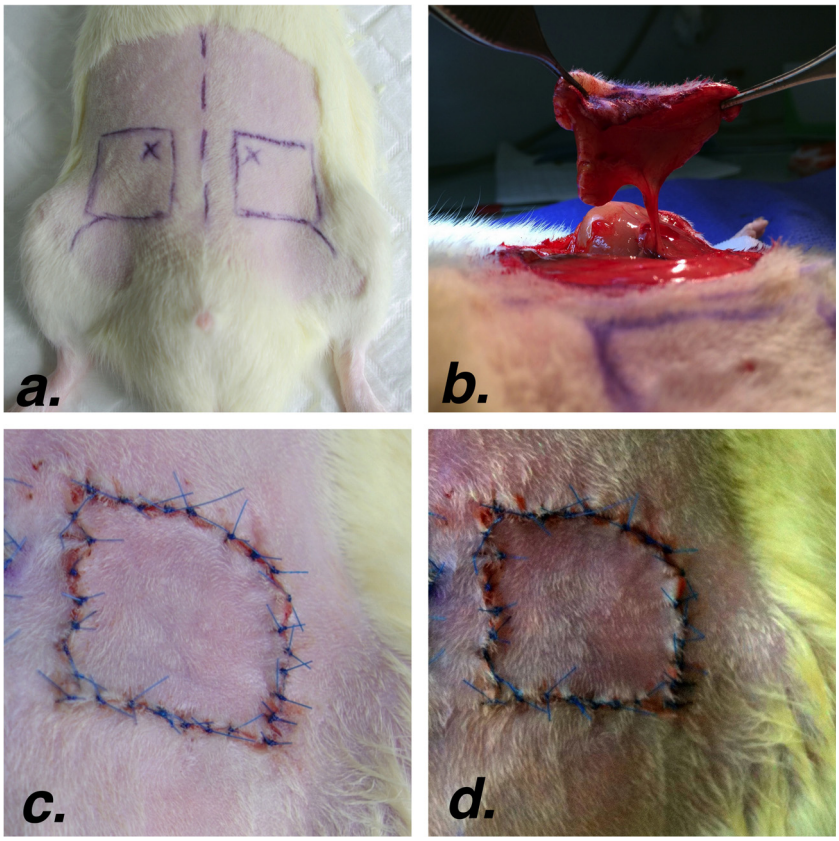

Figure 1 a: tracing of the inguinal flap in a rat; b: flap lifted on the inferior epigastric pedicle; c: aspect of the flap after manipulation; d: aspect of the flap on the third day: pink color, local heat and start of hair regrowth are considered as indicators of successful anastomosis.

In our operation, on the other hand, the vascular pedicle island flap was lifted at the level of the epigastric pedicle (Fig. 1b). Once the epigastric vessels had been dissected, the external diameter of the artery was measured.

The anastomosis technique

Once the inferior epigastric artery (IEA) had been isolated (Fig. 2), it was sectioned after clamping the femoral artery above and below the origin of the IEA. We thereby avoided direct application of the clamps on the IEA itself. Vessel extremities were injected with hyaluronic acid (HA) (Juvéderm Ultra 4®, Allergan, Inc.) by means of a blunt 30 G needle in order to ensure that the vessel lumen would remain open (Fig. 3).

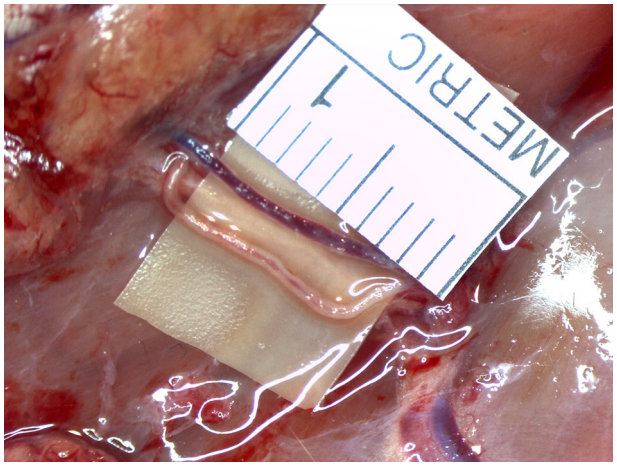

Figure 2 Dissection of the full-length inferior epigastric artery (below) and vein (above).

Following HA injection, termino-terminal anastomoses were carried out by the asymmetric biangulation method with separate supporting sutures (Fig. 4). During this phase, we used a 12—0 round-needled suture (50 µet of 3/8th of circle: S&T®).

Once the anastomosis had been completed, the clamps at the level of the femoral artery were removed (Fig. 5) and the anastomosis was verified in accordance with the O'Brien permeability test, which was applied anew at 15 min so as to confirm the absence of early thrombus formation.

Finally, the flap was sutured in place (Fig. 1c) and daily control was performed until the 3rd postoperative day; local heat level and cutaneous coloration were thereby verified (Fig. 1d).

Data collection

The following data were collected during each manipulation:

- measurement of the external diameter of the inferior epigastric artery (Fig. 6): with 25-fold magnification and a measuring device graduated in millimeters and

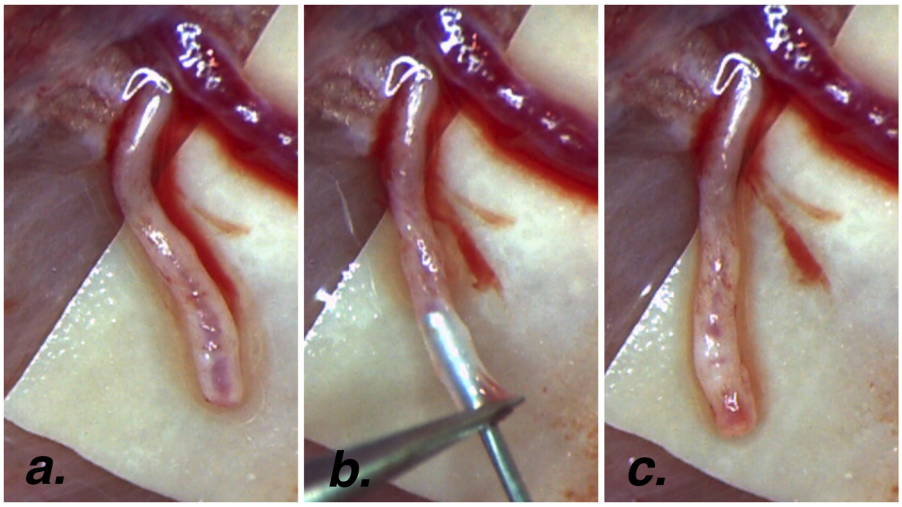

Figure 3 Technique of intravascular injection of hyaluronic acid. a: aspect of the artery before injection; b: introduction of the 30 G blunt-edged needle; c: (whitish) aspect of the artery after injection.

placed in the field of vision, a digital photo was taken. The resulting photographic cliché was transferred onto a computer and external diameter was measured with ImageJ version 1.43u software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij);

- the number of stitches required in each anastomosis;
- anastomosis duration (DA): It was measured in the minutes between IEA sectioning and clamp withdrawal.

Results

The mean diameter of the anastomized arteries was 0.42 mm (min = 0.29 mm; max = 0.47 mm) (Table 1). Mean duration of the anastomosis process was 17.5 min with a minimum of 14 min and a maximum of 21 min. Fig. 7 illustrates duration of anastomosis for each manipulation. The mean number of stitches was 5.9 by anastomosis. The rate of immediate permeability was 100 % (n = 10). Flap survival rate on the 3rd day was 80 % (n = 8). The two flaps considered as failures for animal No.3 were lost through autocannibalism. At D+1 of the daily inspection, we found this animal with 2 losses of substance having replaced the flaps (Fig. 8).

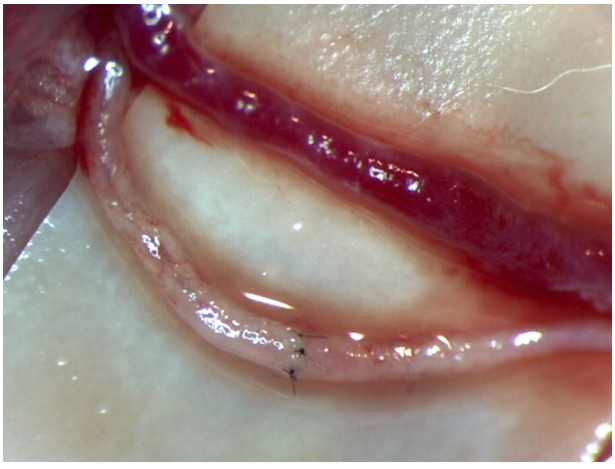

Figure 4 Aspect at end of anastomosis and before unclamping. The whitish aspect is persistent, and it is related to presence in anastomosis of hyaluronic acid.

Discussion

Supermicrosurgery represents a new challenge that is not only technical, but also therapeutic. While new treatment-based strategies have been put forward and appear promising, their worldwide implementation has remained limited to a small number of surgical teams [8]. Even though extensive teaching should pave the way to wider use of supermicrosurgery, what matters most at this time is to develop and apply reliable and reproducible methods. In cases where conventional anastomosis techniques are still applicable, a variety of methods have been proposed [9—12] with the aim of simplifying surgical procedure, but only through techniques facilitating anastomosis procedures is supermicrosurgery likely to finally be democratized.

Two of these techniques, both of which are widely used, present the advantage of avoiding the risks entailed by transfixiant suturing. The first of them requires no specific material but rather consists in a technical modification, and is known as the method of ''open guide suture'' [9]. Stitches are positioned step by step and only subsequently knotted in a precise order, which differs according to involvement of the anterior or the posterior wall of the anastomosis. While this method seems effective and could be rapidly applied in expert hands, it is easy to understand that in practice, the main drawback consists in the numerous stitches ''on standby'' next to vessels of exceedingly small dimensions. The second technique goes by the name of temporary intravascular stent (IVaS) [11]. Since the vascular lumen is obliterated by a foreign body that is withdrawn following stitching, the risk of transfixiant suturing is pronouncedly reduced. Unfortunately, IvaS entails practical drawbacks such as difficulties in manipulating the stent and substantial risks of intimal lesions when the stent is not properly prepared or when there are too many manipulations

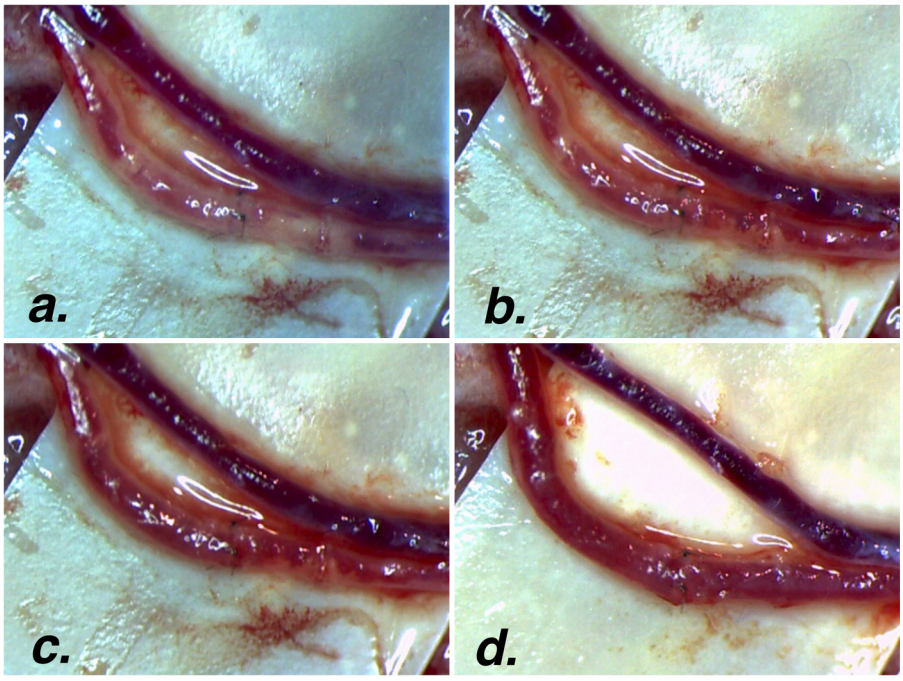

Figure 5 Stages of vascular flow and dissolution of the hyaluronic acid, which takes a few seconds after unclamping. a: vascular flow has been restored and is visible upstream from the anastomosis (on the right); b: the flow has reached the anastomosis; c: the flow has traversed the anastomosis, but as is shown by the irregular aspect of the blood upstream, hyaluronic acid is still present; d: blood flow is now complete, throughout the artery.

to be carried out. Moreover, a possibly steep learning curve may prove detrimental to motivation.

The technique we are proposing is premised on the injection of HA into the vessel lumen, and its main interest consists in the fact that it does not modify microsurgical routine. The separate suturing technique remains the same, and does not necessitate manipulation with a stent. In the literature, hyaluronic acid has recently been suggested as a means of facilitating venous anastomoses [15]. In their study, Yazici et al. [15] proposed that HA be used in end-to-end venous anastomoses of a rat's jugular vein; they were aiming to prevent venous collapse. We are convinced that HA gel becomes particularly interesting as a support for microsurgical anastomoses of vessels with diameters $\leq$ 0.5 mm.

In our work, we chose HA at 24 mg/mL (Juvéderm Ultra 4®, Allergan, Inc.) not only on account of its concentration, but also due to its viscosity, which is high enough [16] to allow it not only to remain in the vascular lumen, but to keep the lumen open. Moreover, product viscosity is in no way incompatible with injection, which was performed without effort at the level of the vascular extremities and enabled us to achieve vascular dilatation that can be maintained throughout the

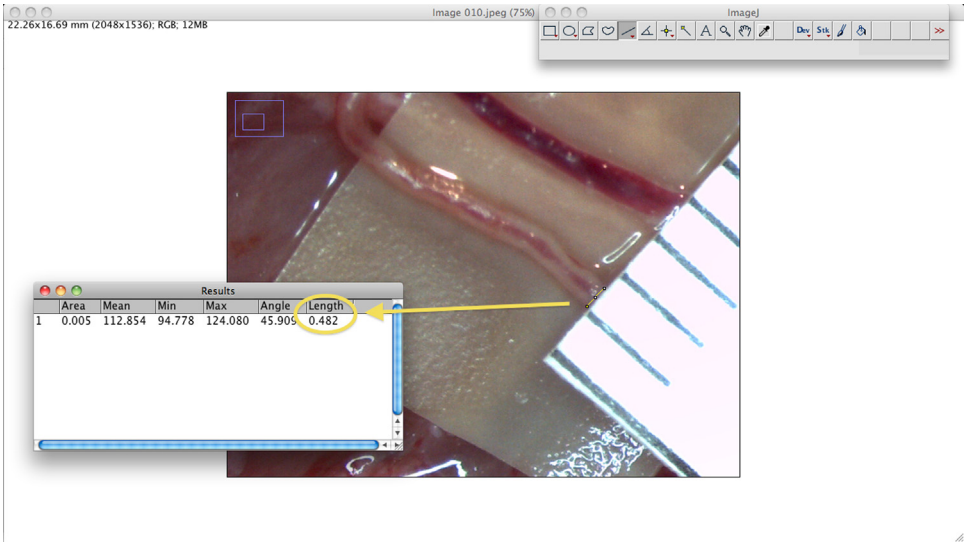

Figure 6 Calculation of inferior epigastric artery (IEA) diameter with ImageJ software.

Table 1 Results of IEA anastomoses..

IEA	Diameter (mm)	Number of sutures	Anastomosis duration (min)		Immediate permeability	D+3
1D	0.42	6	18		1	1
1G	0.48	6	21		1	1
2D	0.45	6	20		1	1
2G	0.46	6	17		1	1
3D	0.29	5	18		1	0
3G	0.35	6	16		1	0
4D	0.45	6	17		1	1
4G	0.42	6	14		1	1
5D	0.44	6	19		1	1
5G	0.47	6	15		1	1
Mean	0.42	5.9	17.5	Success rate	100% (n = 10)	80% (n = 8)

IEA: inferior epigastric artery; 1: success; 0: failure.

anastomosis and minimize the risks that would have been entailed by transfixiant suturing. In addition, anastomosis is less time-consuming than IvAS (mean time: 30 min; maximum: 55 min; minimum: 18 min) [13] and there is no real learning curve. With HA, mean anastomosis duration is 20 min less for vessels inferior to 0.5 mm in diameter. Another benefit consists in a satisfactory patency rate (80% flap survival after 3 days). We did not observe any flap necrosis, either partial or total. The 2 flap failures were secondary to a phenomenon of autocannibalism that commonly occurs in work with rodents [17]. We consider it important to report this event to researchers wishing to engage in work similar to ours and whose eventual results might be biased by a largely avoidable development. In fact, we could and should have prevented what happened; unfortunately, it was only *a posteriori* that we informed ourselves of the prevention methods described in the literature [18—20].

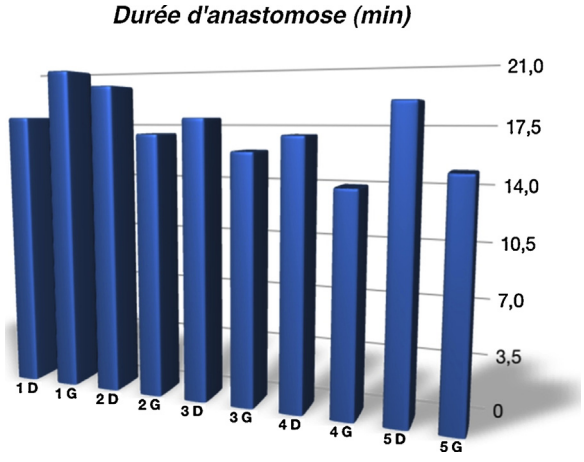

Figure 7 Duration of anastomosis by manipulation.

Figure 8 Surgical site aspect on D+1 for rat No.3.

Let us suppose that the result of our work appear to demonstrate the interest of HA as a promising means of facilitating supermicrosurgical anastomoses. A key question demanding a persuasive response nonetheless remains unanswered: what is the risk entailed by vascular applications of hyaluronic acid fillers? Given the fact that an increasingly high number of cutaneous necroses have been reported following injection of small amounts of HA [21—23], how can it be safely deployed in practically microscopic vessels?

While the exact mechanism of necroses secondary to HA injections has yet to be totally elucidated, two possible causes are frequently put forward [24—26]: intravascular occlusion by accidental intra-arterial injection and extrinsic vascular compression secondary to the volume of HA. In our study, HA is injected into 2 to 3 mm of vessels with a mean diameter of 0.42 mm. On that basis and by equating each vascular extremity to a cylinder, the mean volume injected for each anastomosis can be estimated at $8.4.10^{-4}$mL (V: cylinder volume = $\pi \times r^2 \times h$, with r = 0.42/2 and h = 3 mm, we obtain as a result V = 0.42 mm^3 by vascular extremity, that is to say V_{total} = 0,84 mm^3 by anastomosis; and 1 mm^3 = 0,001 mL). This figure is close to negligible, and we believe it explains why no vascular or cutaneous complications were observed in our study. That much said, now that the feasibility of the method we are proposing has been shown, further work must imperatively involve anatomopathological analyses at the level of the anastomized vessels and the corresponding cutaneous flaps, and they will be meant to prove the absence of short-term or long-term impact on the tissues involved.

Conclusion

We have described an initial utilization of HA in supermicrosurgical anastomosis. Our method has been described in detail, and the results appear promising. Hyaluronic acid effectively simplifies anastomoses of vessels with a diameter less than or equal to 0.5 mm, mean operation time is lower than 20 min, and the patency rate is satisfactory. It is nonetheless indispensable that future studies provide confirmation of these results, and it is especially important that they assess the short and long-term impact of HA on tissues, even when the volumes to be injected are exceedingly low.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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