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ARTICLE

Comparison of total anastomosis time between four different combinations of suturing and knot tying techniques in microsurgical anastomosis

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ABSTRACT

Background: Various techniques have been described for performing microsurgical anastomosis with providing high patency rates. Although the total anastomotic time may not be an issue when dealing with a single set of anastomoses, using a faster technique may save significant amount of time in cases of transferring flaps with shorter critical ischemia time or where multiple anastomoses are required. This study compares the total anastomosis time between four different combinations of commonly used suturing and knot tying techniques.

Methods: Twenty-four rats were divided into 4 groups. Simple interrupted suture with conventional knot tying technique (SIS-CT) was used in group I, continuous suture technique with conventional knot tying (CST) was used in group II, simple interrupted suture with airborne knot tying technique(SIS-AT) was used in group III, and continuous-interrupted suture with airborne knot tying technique(CIS-AT) was used in group IV for microsurgical anastomosis. Total anastomosis time and patency rates with each technique and samples from anastomotic sites were analyzed.

Results: The mean time required for microvascular anastomosis of the femoral artery was 1075 s in group I, 799 s in group II, 844 s in group III, and 973 s in group IV. The difference between four groups was statistically significant. The anastomoses in group II and group III were completed in the shortest period of time. Intergroup comparison revealed that the difference between group II and group III was not statistically significant, however, total anastomosis time for completion of the anastomosis was significantly longer for group I, followed by group IV. Thrombosis rates and histological analysis revealed no significant differences among four groups.

Conclusion: CST and SIS-AT techniques can significantly reduce microsurgical anastomosis time and provide high patency rates. Also, the time needed to complete an anastomosis was significantly shorter for CIS-AT when compared to SIS-CT.

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KEYWORDS
Anastomosis time; airborne suture; critical ischemia; microsurgical anastomosis

Introduction

Advances in microsurgical techniques, instruments, micro sutures and microscopes allow the microsurgeons to successfully perform patent anastomoses of the vessels under 1 mm in diameter and these improvements enabled the overall success rate in free tissue transfers to reach 99% [1]. Constant success in anastomosis patency can be achieved with utilization of the proper technique and this requires specialized training, experience and skills. However, there are certain circumstances where total anastomotic time becomes the major determinant in success of the procedure. The critical ischemic time in bowel flaps (free jejunum, ileocolon and colon), muscle flaps and free flap takebacks is relatively short when compared to the standard microsurgical procedures [2,3]. The transfer of multiple free flaps, supercharging, turbocharging, multiple digit replantation and the use of a vein graft increases the number of anastomoses to be performed and technical anastomotic errors necessitate perioperative revisions of the problematic anastomoses. Besides, intraoperative medical problems may force the surgeon to complete the procedure promptly. Therefore, an ideal anastomotic technique should provide long term patency but also minimize the anastomotic time.

There are still controversial proposals about selecting the best method to maximize patency and shorten the operative time when performing microsurgical anastomosis. The simple interrupted suture technique with conventional knot tying remains the gold standard technique for most of the surgeons [4]. However, this technique is time consuming because of increased number of stitches and the time needed to tie each suture [5]. Alternatively, airborne suture tying technique has been proposed to quickly tie the knots by maintaining the suture end in the air, off the surrounding structures [6]. In order to decrease the number of stitches, continuous suture technique has been developed. This technique allows the surgeon to complete the anastomosis in a very short time, however, it possesses certain disadvantages, such as lumen narrowing with purse-string effect, formation of a rigid

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ring at the anastomosis site and reducing the blood flow distal to the anastomosis, and leaving a greater amount of suture material on the vessel wall which may potentially cause inflammatory response [7,8]. The continuous-interrupted suture technique involves creating several loops and tying the loop ends individually as interrupted [9]. This technique combines advantages of the continuous and interrupted suture techniques. Anastomotic time can be further shortened with utilization of the airborne knot tying technique. There are numerous reports advocating the use of a specific suturing technique in order to complete the anastomosis quickly and with patency [10–14]. Nevertheless, many of these techniques did not gain enough popularity to be performed widely. Ultimately, it is the surgeon's decision to perform a specific technique and it depends on his/her experience, skills and the technique's suitability for that particular case.

In this experimental study, microsurgical anastomoses were performed by a single surgeon on the femoral artery of the rats and total time spent for completing an anastomosis was compared between four different combinations of suturing and tying methods (simple interrupted suture and conventional knot tying technique, continuous suture technique, simple interrupted suture and airborne knot tying technique, continuous-interrupted suture and airborne knot tying technique), and histological analysis was performed to evaluate the effects of suturing techniques on the anastomotic site on the vessel wall thickness.

Material and methods

Permissions were obtained from Research and Ethics Commission for laboratory animal use. Animal care and experiments were complied with the institution's guidelines on care and use of the laboratory animals.

Experimental groups

Twenty-four adult, male Sprague Dawley rats weighing 300–350 g were randomized and divided into 4 groups according to the anastomosis technique. Bilateral femoral arteries of each rat were used for microsurgical anastomosis and total of twelve anastomoses were performed in each group ($n = 12$). Simple interrupted suture with conventional knot tying technique (SIS-CT) was used in group I, continuous suture technique with conventional knot tying (CST) was used in group II, simple interrupted suture with airborne knot tying technique (SIS-AT) was used in group III, and continuous-interrupted suture with airborne knot tying technique (CIS-AT) was used in group IV for microsurgical anastomosis. Total number of stitches and total time for anastomosis was recorded for each technique individually and timing was started with the pass off the first suture and ended with tying the last knot. All animals were kept in 12 h day/night cycle, followed up in plastic cages with sawdust bedding in standardized temperature (24 °C) and humidity environment, and fed *ad libitum*.

Surgical procedure

The rats were anesthetized with intraperitoneal injection of tiletamine hydrochloride and zolazepam hydrochloride (80 mg/kg Zoletil 50, Virbac Corporation, France). Bilateral inguinal regions were shaved and cleaned with an antiseptic solution and the skin was incised using scalpel along the inguinal crease (Figure 1(A)). Inguinal fat pad was exposed and sharply dissected off from the surrounding tissue, between external oblique and adductor muscles. The femoral artery and vein were exposed (Figure 1(B)). The artery was dissected off the vein and the surrounding tissue circumferentially deep branch was ligated and cut, and limited adventitectomy was performed around the future anastomotic site after placing approximator clamps. Sharp microdissection scissors were used to cut the artery where its diameter was recorded as 1 mm with a microscale ruler. Intraluminal irrigation was performed using a diluted heparinized solution to washout remaining blood. Microsurgical anastomosis was completed by using the specific technique using a 10–0 nylon micro suture (Ethilon, Ethicon, United States) and inguinal fat pad was left over the anastomosis for 1 min in order to stop oozing. Early patency was checked using milking test 5 min after the completion of the anastomosis [15]. The inguinal incision was closed primarily with a running non-absorbable suture then, the same procedure was performed on the opposite side. On postoperative day 28, bilateral inguinal scars were re-incised and femoral arteries were exposed. Long-term patency was checked using the milking test and en-block samples from the anastomotic sites were obtained for histological evaluation.

Simple interrupted suture and conventional knot tying technique (group I)

Two stay sutures were placed at 0 and 180 degrees. Simple, interrupted sutures were placed on the opposing vessel ends and knots were tied conventionally (Figure 2). Once suturing of the anterior wall was completed with four additional sutures, the clamp was rotated 180 degrees to suture the back wall in a

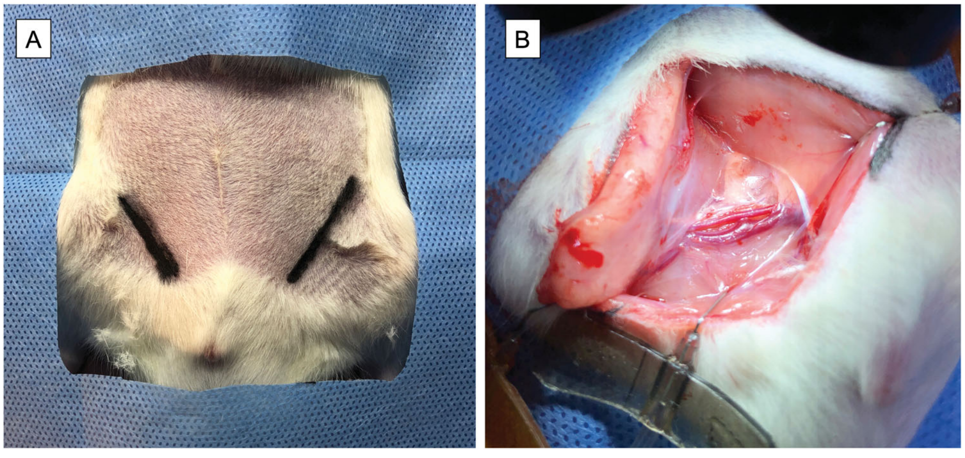

Figure 1. Bilateral inguinal crease incisions (A) were used to expose the femoral artery (B).

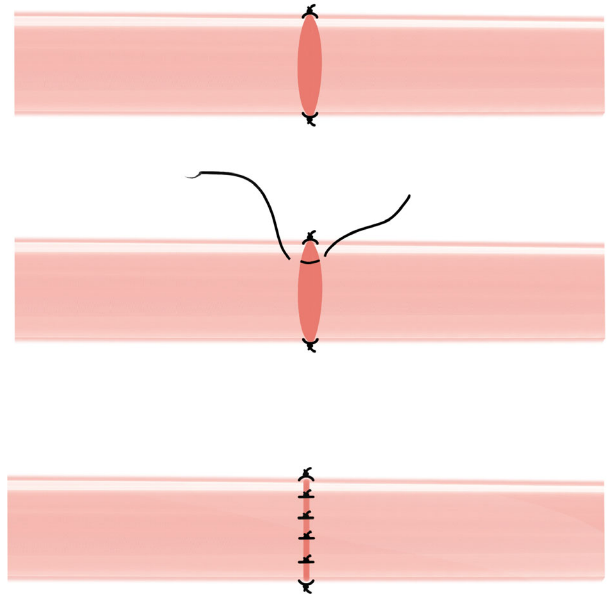

Figure 2. Illustration of the simple interrupted suture technique.

similar manner. In total, ten sutures were placed for completion of the anastomosis.

Continuous suture technique (group II)

The first stay suture was placed at 0 degrees and tied with leaving one end of the thread long. The second stay suture was placed at 180 degrees and tied with leaving one end of the thread long and the other end was used for running suturing the anterior wall. Four passes were performed on the anterior wall and the suture was tied with the long end of the first stay suture (Figure 3). Then, the clamp was rotated 180 degrees and the posterior wall of the anastomosis was performed in a similar manner, tying the final suture with the long end of the second stay suture. All knots were tied by using the conventional knot tying technique.

Simple interrupted suture with airborne knot tying technique (group III)

This technique only differs from SIS-CT group by tying the knots using airborne suture tying technique as described in the literature [6]. In this method, after first pass of the simple interrupted suture by the opposing vessel ends, the suture is pulled through so that the leading suture end is about three times longer than the free end of the thread. The free end is passed behind the opposite end while the right forceps forms the knot and the free end leans on the other segment of the suture. The free end is then picked up at its tip and pulled through the loop and the knot is completed.

Continuous-interrupted suture with airborne knot tying technique (group IV)

The first stay suture was placed at 0 degrees and tied with leaving one end of the thread long. The second stay suture was placed at 180 degrees and tied with leaving one end of the thread long and the other end was used for running the suture at the anterior wall by creating three loose loops. Loops were cut gradually and created free ends were tied with the following thread ends (Figure 4). Once suturing of the anterior wall was completed, the clamp was rotated 180 degrees to suture the back wall in a similar manner. Totally, ten sutures were tied on the

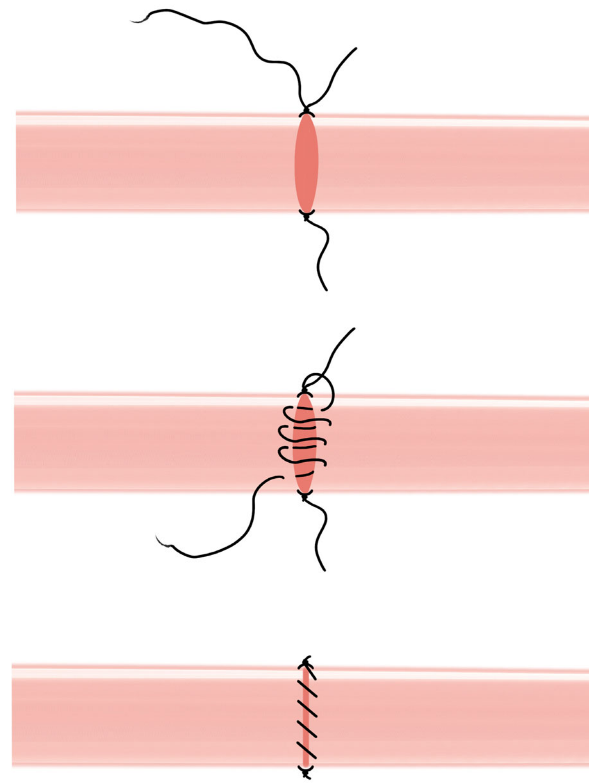

Figure 3. Illustration of the continuous suture technique.

vessel wall by using airborne suture tying technique as described above.

Histological analysis

Samples from the anastomotic sites were fixed in 10% buffered formaldehyde and embedded in paraffin. Sections of 5 µm thickness were obtained at the plane vertical to the anastomosis line and stained with Hematoxylin-Eosin (H&E). Randomly selected areas from each sample were imaged and micrographs were taken at x40 and x100 magnification. Micrographs from all groups were analyzed morphometrically in terms of histological layers of the vessels (tunica intima, tunica media, tunica adventitia) with ImageJ image analysis software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin). For each vessel, the histological layers' area was separately measured and corrected with luminal circumference to avoid miscalculations originating from oblique sections.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences for Windows SPSS 23.0 (IBM Corporation, Armonk, New York, United States). The normal distribution of each group was assessed by the Shapiro-Wilk test. The homogeneity of variances was analyzed with Levene's test. Kruskal-Wallis test was used for comparison of the quantitative data (anastomosis time) of the four groups. Mann-Whitney U test with Bonferroni correction was used for post-Hoc analysis of pairwise comparisons. Fisher Exact test was used to compare the categorical data (outcomes of the anastomoses). Descriptive statistics of quantitative variables were presented as mean ± standard deviation and distribution range. Categorical variables were reported as frequency (percentage) in the tables. For the statistical analysis of the results

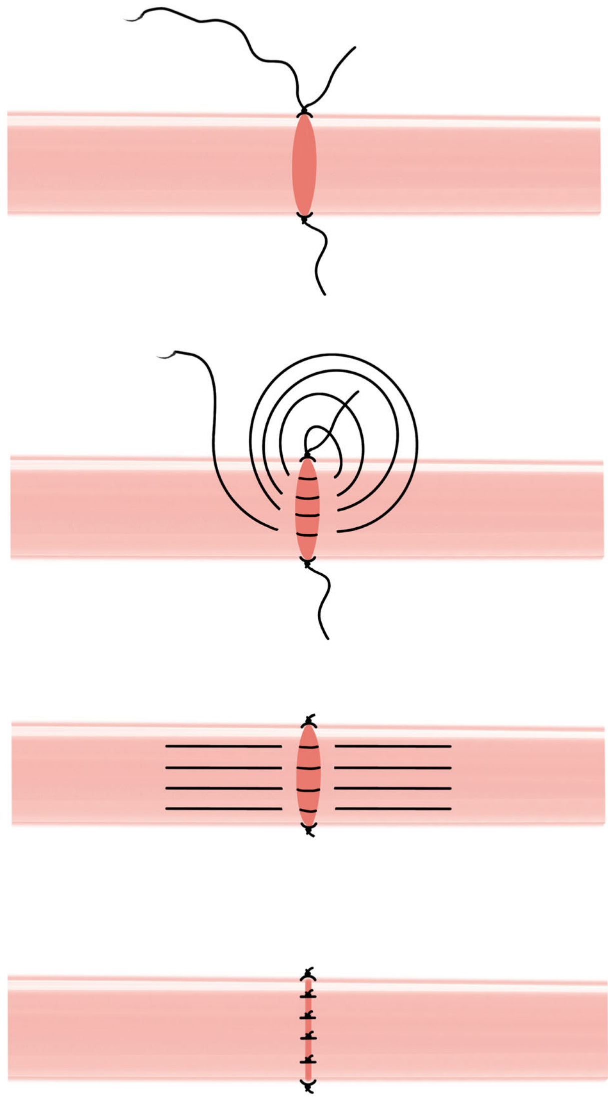

Figure 4. Illustration of the continuous-interrupted suture technique.

obtained from histological evaluation, Prism 8 (GraphPad, California, United States) software was used. Shapiro-Wilk test was used for assessment of normal distribution, and Kruskal-Wallis test was used for comparison of quantitative data. Dunn's test was used for post-Hoc analysis of pairwise comparisons. All of the variables were examined at a 95% confidence level, and *p*-values < 0.05 were considered statistically significant.

Results

Anastomosis time

The mean time required for microvascular anastomosis of the femoral artery was 1075s in group I, 799 s in group II, 844 s in group III, and 973 s in group IV (Table 1). The difference of total anastomosis time between four groups was statistically significant ($p < 0.001$) (Table 2). The anastomoses in group II and group III were completed in the shortest period of time ($p < 0.001$). Intergroup comparison revealed that the difference between group II and group III was not statistically significant ($p = 0.056$), however, total anastomosis time for completion of the anastomosis was significantly longer for group I, followed by group IV ($p = 0.004$).

Table 1. Comparison of the anastomosis time between four groups.

	Anastomosis time (seconds)		
	Mean ± SD	Range	*p* Value
Group I	1075.41 ± 74.27	970 – 1170	**<0.001**[a]
Group II	799.58 ± 49.01	715 – 870	
Group III	844.17 ± 57.40	740 – 920	
Group IV	973.33 ± 54.41	860 – 1030	

[a]Kruskal-Wallis test. SD: Standard deviation.

Table 2. The *p*-values of pairwise comparisons between four groups[a].

Groups	I-II	I-III	I-IV	II-III	II-IV	III-IV
p-value	**<0.001**	**<0.001**	**0.004**	0.056	**<0.001**	**<0.001**

[a]Post-Hoc Mann-Whitney U test with Bonferroni correction.

Thrombosis rates

Milking test revealed %100 patency in all groups, 5 min after completion of the anastomosis. One artery (1/12) from group II and one artery (1/12) from group III were found to be occluded on postoperative day 28, yielding a patency rate of 91.7% for both groups. All of the anastomoses were patent (100%) in group I and group IV. Intergroup comparison revealed no statistically significant difference between four groups regarding patency rates ($p = 1.00$) (Table 3).

Histological analysis

Evaluation of tunica intima and tunica media thicknesses revealed no statistically significant difference among four groups ($p = 0.56$ and $p = 0.99$, respectively). Tunica adventitia thickness was significantly increased in group III and group IV when compared to group I ($p = 0.03$ and $p = 0.02$, respectively), however, this difference was attributed to the small sub-adventitial hematomas which were observed in the analyzed micrographs and interfered with the measurements. There was no further difference in the general structure of the vessel walls among four groups (Figure 5).

Discussion

The ideal microsurgical anastomosis technique would be easy to perform and teach, avoid trauma to the vessel wall, minimize ischemia time, reduce operative time and costs, and provide long-term patency. Several alternative techniques have been described in order to reduce the total time required to perform the microsurgical anastomosis with providing high patency rates at the same time; including non-suturing techniques, such as using coupler, laser, staples, adhesive glue and intravascular stent [4,16–20]. Couplers and hand-sewn anastomosis techniques are both employed more often, however, couplers come with a greater expense when compared to the micro sutures and the risk of complications increase with the use of smaller couplers under 2.0 mm in diameter [21,22]. Therefore, hand-sewn anastomosis techniques remain the most commonly preferred choice in performing microsurgical anastomosis. Which of the various suturing or knot tying techniques is superior is often a reason for debate, and the decision of using a specific technique mostly depends on surgeon's own experience and preference. Although the total anastomotic time may not be an issue when dealing with a single set of anastomoses, using a faster technique may save significant amount of time in cases of transferring flaps with shorter critical ischemia time or where multiple anastomoses are required. Completing a microvascular anastomosis in a shorter time decreases the risk of ischemic injury and vessel wall trauma, total operative time, and finally contributes to the overall success of the microsurgical procedure [14].

The most common method for performing and teaching microsurgical anastomosis is the simple interrupted suture technique with conventional knot tying [4]. However, this technique requires tying many knots and repetitive refocusing of the magnified view, which lengthen the overall anastomosis time when compared to the continuous suture technique and its modifications [7,11,14,23,24]. In accordance with the findings of previous reports, this suturing technique was found to be slower than CST and it was proven for the first time with quantitative data that this technique requires longer time when compared to CIS-AT and SIS-AT.

Continuous suture technique utilizes a running suture over the vessel wall, obviating the need for multiple knot tying. Chen et al. has reported that simple continuous suture technique decreases the microvascular arterial anastomosis time by 50% when compared with the simple interrupted suture technique [8]. In our study, CST was shown to be a faster technique than SIS-CT and CIS-AT, but the total time needed to complete an anastomosis was similar with SIS-AT. Nevertheless, continuous suture technique has the potential disadvantage of formation of a rigid ring around the anastomotic site with excessive constriction of sutures causing stenosis and decrease in blood flow distal to the anastomosis [10]. If perioperative revision of the anastomosis is needed, entire suture should be taken down and this would reverse the time advantage. Also, elimination of the vessel size discrepancy is troublesome with using this technique. Therefore, continuous suture technique in anastomosis of small vessels is rarely recommended. This technique leaves a greater amount of suture material around the anastomosis site when compared to the interrupted suture technique, and increase in severity of

Table 3. Comparison of the anastomosis patency rates on POD 28.

	Outcome *n* (%)	
	Patent	Occluded
Groups		
I	12 (100.0)	0 (0.0)
II	11 (91.7)	1 (8.3)
III	11 (91.7)	1 (8.3)
IV	12 (100.0)	0 (0.0)
p-Value[a]	1.00	

[a]Fisher Exact test, POD: postoperative day.

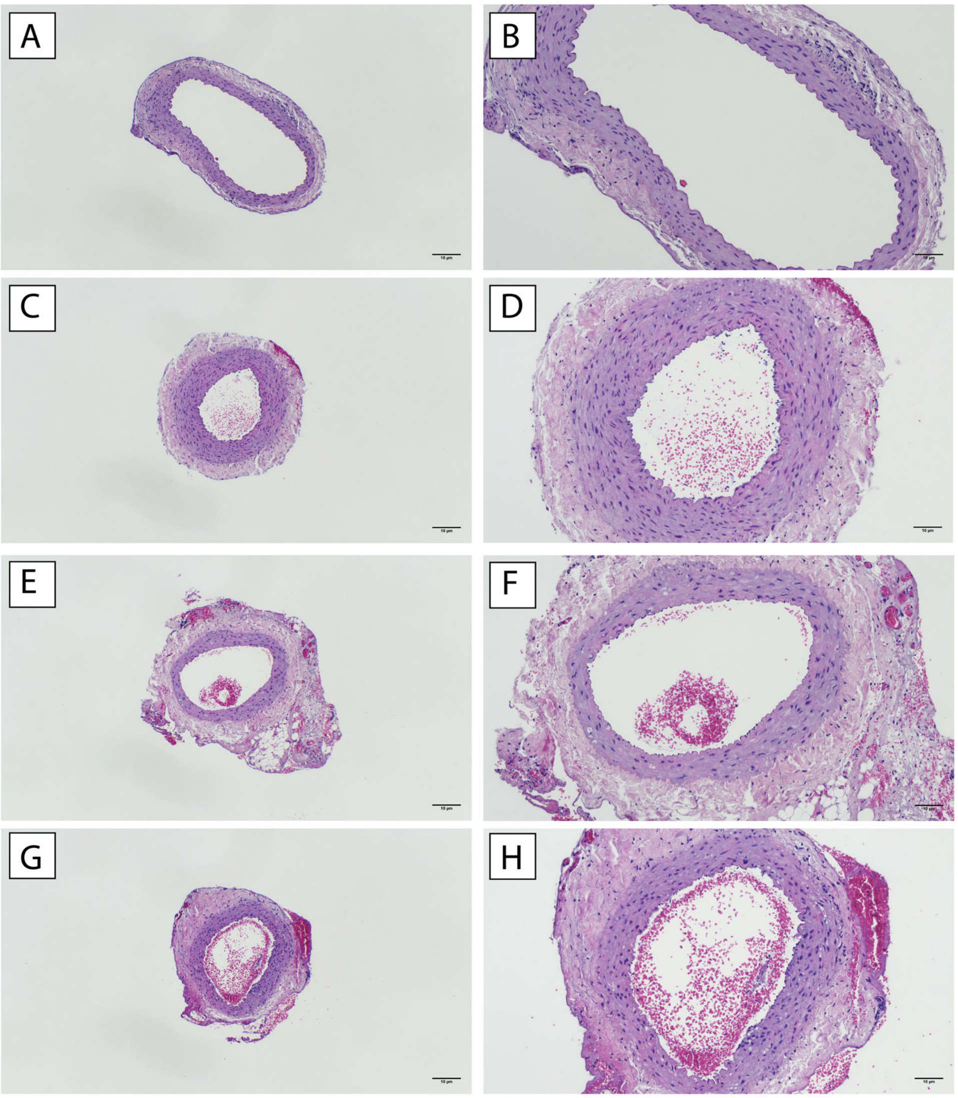

Figure 5. Histological evaluation revealed similar healing process and vessel wall thickness among four groups (Hematoxylin-Eosin staining, x40 and x100 magnification, 10 μm scalebar).

inflammation and the risk of thrombosis may be hypothesized with increased contact of the suture material with the vessel wall. However, histological analysis of our study revealed no significant difference in acute inflammation parameters and thrombosis rates between four groups.

Airborne knot tying method has been developed in order to alleviate the cumbersome process of picking the suture off the surrounding structures, which may lead to unnecessary manipulation of the adventitia and surgeon frustration, as confronted in conventional knot tying technique [25]. In concordance with the experiences of Chen et al., it was proven that this technique can save approximately 15–20% on total anastomosis time when used instead of the conventional knot tying method [6]. Moreover, it was proven in our study that this knot tying method provides a comparably fast anastomosis time with avoiding the risks related to CST. This method is applicable not only to the simple interrupted suture technique but also any alternative suturing techniques as used in conjunction with continuous-interrupted suture technique in group IV. Cigna et al. have advocated combining the airborne technique with posterior wall first, continuous-interrupted suture technique and the authors concluded that this combination results in significantly shorter microsurgical operative time when compared to the conventional method [5]. Onoda and Masahito have reported the benefits of airborne technique when utilized in lymphaticovenular anastomosis; the average operative time associated with the airborne technique was observed to be approximately three-fourths of the operative time associated with the conventional knot tying procedure [26].

Continuous-interrupted suture technique combines the advantages of interrupted suturing and continuous suturing; placing a running suture shortens the anastomotic time, the vessel lumen can be visualized throughout the anastomosis and anastomotic stenosis is not an issue as the sutures are tied individually after cutting the loops. In previous reports, total anastomotic time has been compared between several modifications of this technique and the interrupted suture technique with conventional knot tying method. Although the authors advocated the speed of this technique, it was shown to take longer time when compared to CST and total saved time differed in various reports [27,28]. In our study, airborne knot tying method was incorporated to continuous-interrupted suture technique in order to shorten the anastomosis time. It was proven that, this modification (CIS-AT) required shorter time for completion of the anastomosis when compared to SIS-CT, however, it was slower than the CST and SIS-AT. This technique requires more expertise than SIS-CT and CST, as creating and maintaining loose loops and identifying each suture end after cutting the loops are challenging.

Histological outcomes of this study verified that all four groups showed similar healing process and vessel wall thicknesses and there was no statistically significant difference regarding these parameters. All groups had similar long-term patency rates, which also showed no statistically significant difference.

We believe that, several minutes of time savings gained in the experimental design would be reflected to the operation room more obviously. Utilization of a fast and reliable anastomosis technique would be helpful to the microsurgeon to improve microsurgical success. The disadvantage of airborne technique is its relatively longer learning curve, as this method should be mastered in order to be able to observe obvious time savings.

The limitation of our study is that only arterial anastomosis with perfectly matched lumens was included. Superior results with SIS-AT over CST might be observed when dealing with vessel size discrepancy or veins which have more fragile walls against handling. More clinical and experimental data is needed to prove this.

Conclusion

In summary, the results indicate that both CST and SIS-AT techniques can significantly reduce microsurgical anastomosis time and provide high patency rates. Also, the time needed to complete an anastomosis was significantly shorter for CIS-AT when compared to SIS-CT. The authors advocate combining the airborne knot tying method with any suturing technique when performing a microsurgical anastomosis.

Disclosure statement

The authors report there are no competing interests to declare.

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