

1 Experimental study of microvascular anastomosis: Comparison of the conventional method, back
2 wall technique, and untied stay suture method

3

4 **Abstract**

5 **Background**

6 Compared with the conventional microvascular anastomosis method, the back wall technique and
7 untied stay suture method allow for better visualization of the vascular lumen and have been
8 reported to be effective for beginner surgeons. However, there are no reports of such advantages
9 from experimental studies. The present study compared the usefulness of the conventional
10 method (Method C), back wall technique (Method B), and untied stay suture method (Method U)
11 in rats.

12 **Methods**

13 Ninety end-to-end anastomosis procedures of the right femoral artery and vein were performed in
14 rats. The anastomosis condition was assessed at the completion of suturing one side, at the
15 completion of vascular anastomosis, and on postoperative day 7.

16 **Results**

17 After suturing one side, suture errors were observed in three veins with Method C. Immediately
18 after the completion of vascular anastomosis, blood flow was impaired in one vein with Method
19 C. On postoperative day 7, blood flow was impaired in one artery and one vein with Method C,
20 one vein with Method B, and one artery with Method U.

21 **Conclusions**

22 No significant differences were observed between back wall technique and the untied stay suture
23 method. However, the conventional method was more likely to lead to suture error or impaired
24 blood flow compared with back wall technique and the untied stay suture method.

25

26 **Key words:** Microsurgery; Microvascular anastomosis; Vascular end-to-end anastomosis

27 **Introduction**

28 Microvascular anastomosis began in 1960 with the successful anastomosis of a 1.4-mm-diameter
29 blood vessel under a surgical microscope by Jacobson and Suarez.¹⁾ Currently in Japan, the
30 typical microvascular anastomosis methods use two stay sutures, as described by Acland et al.
31 (conventional method),²⁻⁴⁾ posterior wall first anastomotic technique (back wall technique),^{5,6)} or
32 the untied stay suture method,^{7,8)} which was devised as a method to anastomose blood and
33 lymphatic vessels that have external diameters of 0.3-0.5 mm or less.

34 The present study compared the usefulness of the conventional method (Method C), back wall
35 technique (Method B), and untied stay suture method (Method U) as microvascular anastomosis
36 methods in an experimental study involving rats.

37

38 **Methods**

39 The surgeons completed Microsurgery Stage 5, which is the final stage of a microsurgery training
40 program⁹⁾ and had clinical microsurgery experience of 2 years (Surgeon A), 6 years (Surgeon B),
41 and 20 years (Surgeon C). Based on the classifications reported by Tang and Gidding¹⁰⁾, Surgeon
42 A is Level 2, Surgeon B is Level 3, and Surgeon C is Level 4. Forty-five Wistar rats (10-week-old
43 male rats; 230-280 g) were used. Three-type mixed anesthesia¹¹⁾ was used for anesthesia. Each
44 surgeon performed vascular anastomosis using the conventional method (Figure 1), back wall
45 technique (Figure 2), and untied stay suture method (Figure 3) in five animals per method,
46 resulting in end-to-end anastomosis of the right femoral artery and vein in a total of 15 animals.
47 Ninety end-to-end anastomoses of the right femoral artery and vein (external diameter of
48 approximately 1.0 mm) were performed in a total of 45 animals by the three surgeons (Figure 4).

49 The time required for artery and vein anastomoses was measured, and the anastomosis condition
50 at the completion of suturing one side, completion of vascular anastomoses, and on postoperative

51 day 7 were assessed. This study protocol was approved by the Animal Care and Use Committee
52 of the author's institution (No. 16-068) and was conducted based on the institution's Animal
53 Experiment Policy.

54 For statistical analyses, Friedman's test was used for the time required for vascular anastomosis,
55 and Fisher's exact test was used to compare suture error and impaired blood flow. $P < 0.05$ was
56 considered significant.

57

58 **Results**

59 There were no deaths caused by anesthesia or the surgical procedure, and no animals died during
60 observation.

61 The mean durations of artery and vein anastomoses for Method C, Method B, and Method U
62 were: 28 minutes 33 seconds, 30 minutes 8 seconds, and 29 minutes 9 seconds, respectively, for
63 Surgeon A; 20 minutes 16 seconds, 20 minutes 46 seconds, and 21 minutes 12 seconds,
64 respectively, for Surgeon B; and 22 minutes 47 seconds, 23 minutes 4 seconds, and 23 minutes 46
65 seconds, respectively, for Surgeon C.

66 Surgeon B was the fastest, and Surgeon A was the slowest for all methods when evaluating the
67 mean duration of anastomosis for artery and vein by surgeon (Table 1).

68 For all surgeons, Method C was performed the fastest when evaluating the mean duration of
69 anastomoses for artery and vein by method ($P = 0.0094$ for Friedman's test) (Table 1).

70 In the assessment of anastomosis condition of the 90 anastomoses, suture errors at three veins
71 were observed with Method C at the completion of suturing one side. Immediately after
72 completion of vascular anastomosis, impaired blood flow of one vein was observed with Method
73 C. On postoperative day 7, impaired blood flow was observed at one artery and one vein with
74 Method C, one vein with Method B, and one artery with Method U (Table 2).

75 Assessment of suture error and impaired blood flow by surgeon showed errors at two vein
76 sutures for Surgeon A and one vein suture for Surgeon C after suturing one side. However,
77 significant differences between surgeons were not observed at this time point with Fisher's exact
78 test (Table 3).

79 At completion of anastomosis, impaired blood flow of one vein by Surgeon A was observed, but
80 this was also not significantly different among the surgeons (Table 3).

81 On postoperative day 7, one artery with impaired blood flow was observed for Surgeons A and
82 B and one vein with impaired blood flow was observed for Surgeons B and C, although there
83 were no significant differences among the surgeons (Table 3).

84 Combined intraoperative assessment after suturing one side and at completion of anastomosis
85 showed that, intraoperatively, none of the surgeons had suture error or impaired blood flow
86 during artery anastomosis, and Surgeon A had three and Surgeon C had one suture error or
87 impaired blood flow during vein anastomosis; however, there were no significant differences
88 among the surgeons (Table 4).

89 Furthermore, the addition of postoperative day 7 data showed that suture errors and impaired
90 blood flow were observed in one artery for Surgeon A, one artery for Surgeon B, three veins for
91 Surgeon A, one vein for Surgeon B, and two veins for Surgeon C. However, there were no
92 significant differences among the surgeons (Table 4).

93 Assessment of suture error and impaired blood flow by method showed errors of three vein
94 sutures with Method C after suturing one side; however, significant differences were not
95 observed among the methods (Table 5).

96 At the completion of anastomosis, blood flow was impaired at one vein anastomosis with
97 Method C, but significant differences were not observed among the methods (Table 5).

98 On postoperative day 7, impaired blood flow was observed at one artery with Method C, one

99 artery with Method U, one vein with Method C, and one vein with Method B; however, no
100 significant differences were observed among the methods (Table 5).

101 Combined intraoperative assessment after suturing one side and at completion of anastomosis
102 showed that suture error and impaired blood flow were not observed at artery anastomoses.
103 However, suture error and impaired blood flow were observed at four vein anastomoses with
104 Method C, a significant difference with Fisher's exact test (P-value=0.027; P-value=0.032 when
105 artery and vein were combined). The results therefore showed that anastomosis error and blood
106 flow impairment are more likely to occur with Method C than with Method B and Method U
107 (Table 6).

108 Furthermore, results with the addition of postoperative day 7 data showed that there were suture
109 errors or impaired blood flow of one artery with Method C and one artery with Method U, and
110 five veins with Method C and one vein with Method B. This resulted in a significant difference in
111 vein anastomosis among the methods with Fisher's test (P-value=0.035); the P-value was 0.045
112 when artery and vein were combined (Table 6). It was also shown that anastomosis error and
113 blood flow impairment are more likely to occur with Method C compared with Method B and
114 Method U, though with a lesser degree of significance.

115 Based on the above, with the assessment of anastomosis condition, there were no significant
116 differences between Method B and Method U, but suture error and impaired blood flow were
117 more likely to occur with Method C than with Method B and Method U.

118

119 **Discussion**

120 The characteristics of each anastomosis method are that, after placing two stay sutures with the
121 conventional method,²⁻⁴⁾ a front wall suture and back wall suture are added (Figure 1). In Japan,
122 the conventional method is the first basic microvascular anastomosis method that is learned by

123 beginners. It is also taught first in our microsurgery training program, where pupils learn the
124 basic operation of forceps, needle holder, and needles.^{3,4,9)}

125 The back wall technique^{5,6)} involves passing the first suture through the back wall and is a
126 method of suturing from back to front in order (Figure 2). The characteristics of the back wall
127 technique are that the lumen can be visualized easily because the sutures start from the back wall,
128 and that it involves a smaller chance of accidentally hooking the needle on the contralateral wall.
129 Moreover, it does not require rotation of the blood vessel during anastomosis. In clinical practice,
130 because it is not necessary to rotate the blood vessel during the procedure, it is useful in vascular
131 anastomosis where the field of view is deep and narrow.⁶⁾

132 The untied stay suture method^{7,8)} involves passing through the first suture at a site that is easiest
133 at the front wall and uses a clip for holding the stay suture instead of tying (Figure 3). The
134 characteristics of the untied stay suture method are that the lumen can be easily visualized
135 because the stay suture is left behind in the vascular lumen without tying, and that the blood
136 vessel only needs to be rotated 90 degrees during anastomosis. Moreover, by applying tension on
137 the first and second stay sutures without tying allows for an easier anastomosis maneuver of the
138 dilated vascular lumen. In clinical practice, it has been reported to be useful for anastomosis of
139 blood vessels and lymph vessels with external diameters of 0.3-0.5 mm or smaller.⁷⁾

140 Back wall technique and untied stay suture methods have been reported to be effective for
141 beginners because the vascular lumen can be visualized well.^{5,7)} However, this has not been
142 reported from experimental studies.

143 In 2015, we started a Microsurgery Training Program (MTP). The MTP starts with anastomosis
144 of artificial blood vessels in Stage 1 and concludes with supermicrosurgery in Stage 5. With the
145 completion of Stage 4, surgeons are given the opportunity for the first time in clinical practice to
146 perform microvascular anastomosis.⁹⁾

147 To match the surgical technique level of the participating surgeons, only those who have
148 completed the Stage 5 supermicrosurgery training were included. The three surgeons had clinical
149 microsurgery experience of 2 years, 6 years, and 20 years.

150 We had predicted that the surgeon with the longest clinical experience would require the shortest
151 amount of time for anastomosis; however, the mean duration of anastomosis for arteries and
152 veins for all methods was fastest by Surgeon B who had 6 years of clinical experience, followed
153 by Surgeon C (20 years of clinical experience) and Surgeon A (2 years of clinical experience).
154 This is perhaps because surgeons who have completed Stage 5 have their own consistent pace in
155 the speed of vascular anastomosis, indicating that clinical microsurgery experience does not
156 necessarily correlate with anastomosis duration for arteries and veins.

157 For all surgeons, the mean duration of vascular anastomosis by method was fastest for Method
158 C, which is the first method to learn as the basics of microsurgery. The order from fastest to
159 slowest was Method C, Method U, and Method B for Surgeon A, and Method C, Method B, and
160 Method U for Surgeons B and C. Method B required a longer time than Method C, likely due to
161 difficulties in suturing and tying procedures at the back wall.⁵⁾ Moreover, the anastomosis
162 duration is longer with Method U compared with Method C, likely because Method U involves
163 additional steps compared with Method C, where the first and second stay sutures are held with a
164 vascular clip rather than tying, and additional time is required to use them as traction thread.

165 There were no significant differences between surgeons at any assessment time point in suture
166 errors and impaired blood flow. This is likely because, while there were differences in clinical
167 experience (2 years, 6 years, and 20 years), the surgeons had acquired sufficient microvascular
168 anastomosis skills through the microsurgery training program and completing Stage 5.

169 Assessment of suture error and impaired blood flow by method showed that these issues were
170 more likely to occur with Method C compared with Method B and Method U. Suture error and

171 impaired blood flow are less likely to occur with Method B and Method U than with Method C,
172 because Method B involves suturing from the back wall and can attain satisfactory visualization
173 of the vascular lumen.^{5,6)} Moreover, Method U involves pulling the first and second stay sutures
174 also used in Method C without tying and proceeds with the vascular anastomosis procedure in a
175 condition where the surgeon can easily confirm the vascular lumen.⁷⁾ These indicate that both
176 methods ensure satisfactory visualization of the vascular lumen. Suture errors of three veins were
177 observed with Method C after suturing one side (Table 2); however, in all cases, the needle was
178 hooked to the contralateral wall adjacent to the stay suture. The possible reason for this is that, by
179 tying the stay suture, the distance between the back wall and front wall had shortened.

180 When comparing the anastomosis method of end-to-end anastomosis, rather than combining
181 intraoperative assessment (after suturing one side + at completion of anastomosis) with
182 postoperative day 7 data, the intraoperative assessment (after suturing one side + at completion of
183 anastomosis) alone showed greater significance (Table 4, Table 6). This is likely because
184 postoperative resting cannot be maintained in rats, resulting in thrombus formation at the
185 anastomosis site, thereby affecting the assessment on postoperative day 7.

186

187 **Acknowledgements**

188 The author would like to thank Professor with special assignment Kenjiro Hasegawa at the
189 Department of Orthopedics, Hand and Reconstructive Microsurgery and Professor Toru
190 Hasegawa at the Department of Orthopedics, Traumatology and Spine Surgery, both at the
191 author's institution, for their advice in preparing this manuscript. The author would also like to
192 thank Lecturer Dr. Kazuhide Okei at the Department of Natural Sciences at the author's
193 institution for his assistance with the statistical analysis.

194

195 **Funding:** This research received no specific grant from any funding agency in the public,
196 commercial, or not-for-profit sectors.

197

198 **Disclosure of conflict of interest**

199 The author has no conflict of interest to disclose in relation to this study.

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228 **Tables**

229 Table 1. Mean Duration of Vascular Anastomosis by Surgeon

	Method C	Method B	Method U
Surgeon A	28 min 33 sec	30 min 08 sec	29 min 09 sec
Surgeon B	20 min 16 sec	20 min 46 sec	21 min 12 sec
Surgeon C	22 min 47 sec	23 min 04 sec	23 min 46 sec

230

231 Table 2. Suture Error and Impaired Blood Flow by Anastomosis Condition

After suturing one side															
	Method C					Method B					Method U				
Surgeon A	A · V	A · X	A · V	A · X	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V
Surgeon B	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V
Surgeon C	A · X	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V
At completion of anastomosis															
	Method C					Method B					Method U				
Surgeon A	A · V	A ·	A · X	A ·	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V
Surgeon B	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V
Surgeon C	A ·	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V
Postoperative day 7															
	Method C					Method B					Method U				
Surgeon A	A · V	A ·	A ·	A ·	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	X · V	A · V	A · V
Surgeon B	X · X	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · V
Surgeon C	A ·	A · V	A · V	A · V	A · V	A · V	A · V	A · V	A · X	A · V	A · V	A · V	A · V	A · V	A · V
A: Artery	V: Vein		X : Confirmed suture error or impaired blood flow												

232

233

234 Table 3. Suture Error and Impaired Blood Flow by Surgeon (1)

After suturing one side

	Suture error and impaired blood flow	Surgeon A	Surgeon B	Surgeon C	P-value
Artery	–	15	15	15	1
	+	0	0	0	
Vein	–	13	15	14	0.762
	+	2	0	1	
Artery + Vein	–	28	30	29	0.77
	+	2	0	1	

At completion of anastomosis

	Suture error and impaired blood flow	Surgeon A	Surgeon B	Surgeon C	P-value
Artery	–	15	15	15	1
	+	0	0	0	
Vein	–	12	15	14	0.309
	+	1	0	0	
Artery + Vein	–	27	30	29	0.321
	+	1	0	0	

Postoperative day 7

	Suture error and impaired blood flow	Surgeon A	Surgeon B	Surgeon C	P-value
Artery	–	14	14	15	1
	+	1	1	0	
Vein	–	12	14	13	1
	+	0	1	1	
Artery + Vein	–	26	28	28	1
	+	1	2	1	

236 Table 4. Suture Error and Impaired Blood Flow by Surgeon (2)

After suturing one side + at completion of anastomosis

	Suture error and impaired blood flow	Surgeon A	Surgeon B	Surgeon C	P-value
Artery	–	15	15	15	1
	+	0	0	0	
Vein	–	12	15	14	0.302
	+	3	0	1	
Artery + Vein	–	27	30	29	0.318
	+	3	0	1	

After suturing one side + at completion of anastomosis + postoperative day 7

	Suture error and impaired blood flow	Surgeon A	Surgeon B	Surgeon C	P-value
Artery	–	14	14	15	1
	+	1	1	0	
Vein	–	12	14	13	0.857
	+	3	1	2	
Artery + Vein	–	26	28	28	0.722
	+	4	2	2	

238 Table 5. Suture Error and Impaired Blood Flow by Method (1)

After suturing one side

	Suture error and impaired blood flow	Method C	Method B	Method U	P-value
Artery	–	15	15	15	1
	+	0	0	0	
Vein	–	12	15	15	0.099
	+	3	0	0	
Artery + Vein	–	27	30	30	0.103
	+	3	0	0	

At completion of anastomosis

	Suture error and impaired blood flow	Method C	Method B	Method U	P-value
Artery	–	15	15	15	1
	+	0	0	0	
Vein	–	11	15	15	0.28
	+	1	0	0	
Artery + Vein	–	26	30	30	0.31
	+	1	0	0	

Postoperative day 7

	Suture error and impaired blood flow	Method C	Method B	Method U	P-value
Artery	–	14	15	14	1
	+	1	0	1	
Vein	–	10	14	15	0.725
	+	1	1	0	
Artery + Vein	–	24	29	29	0.68
	+	2	1	1	

239 Table 6. Suture Error and Impaired Blood Flow by Method (2)

After suturing one side + at completion of anastomosis

	Suture error and impaired blood flow	Method C	Method B	Method U	P-value
Artery	–	15	15	15	1
	+	0	0	0	
Vein	–	11	15	15	<u>0.027</u>
	+	4	0	0	
Artery + Vein	–	26	30	30	<u>0.032</u>
	+	4	0	0	

After suturing one side + at completion of anastomosis + postoperative day 7

	Suture error and impaired blood flow	Method C	Method B	Method U	P-value
Artery	–	14	15	14	1
	+	1	0	1	
Vein	–	10	14	15	<u>0.035</u>
	+	5	1	0	
Artery + Vein	–	24	29	29	<u>0.045</u>
	+	6	1	1	

240 **Figure legends**

241 Figure 1. Conventional method

242 The first suture is passed through (a) and is tied as a stay suture (b), and the second stay suture on
243 the contralateral side is tied (c). At this stage, three sutures are added to the front wall to conclude
244 the front wall suture, and the blood vessel is rotated using the stay suture (d). Subsequently, the
245 back wall is sutured, and the vascular anastomosis is completed.

246

247 Figure 2. Back wall technique

248 The first suture is passed through the back wall (a) and is tied together as a stay suture (b).
249 Subsequently, suturing is continued from back to front in order (c). Completed suture of the back
250 wall (d). Vascular anastomosis is completed after suturing the front wall.

251

252 Figure 3. Untied stay suture method

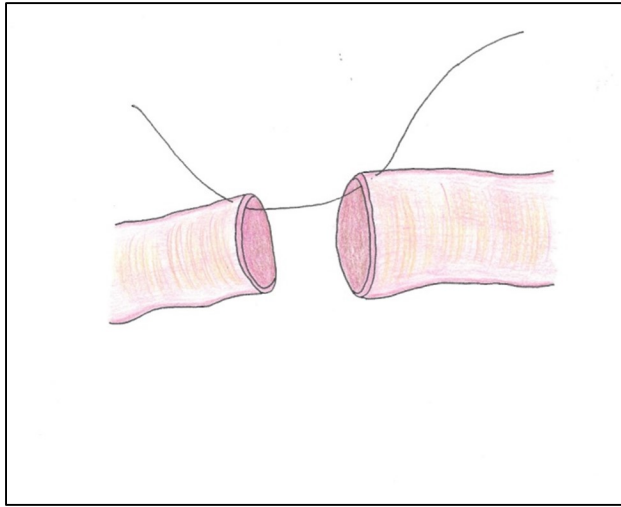
253 The first suture is passed through the front wall, wherever is easiest (a), and the stay suture is
254 held with a clip without tying (b). Another stay suture is passed through the contralateral side and
255 held with a clip without tying (c). At this stage, the front wall is sutured and rotated 90 degrees
256 (d). Subsequently, the back wall is sutured, and vascular anastomosis is completed.

257

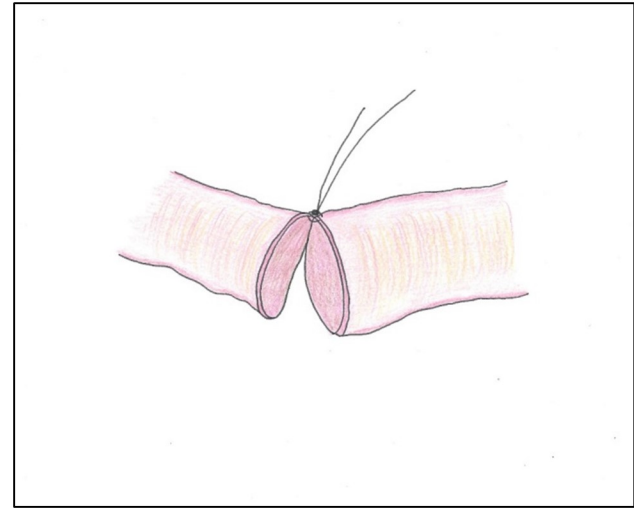
258 Figure 4. Femoral artery and vein of a rat

259 (a) Skin incision to the rat is shown, (b) Femoral artery (external diameter of approximately 1.0
260 mm) before anastomosis

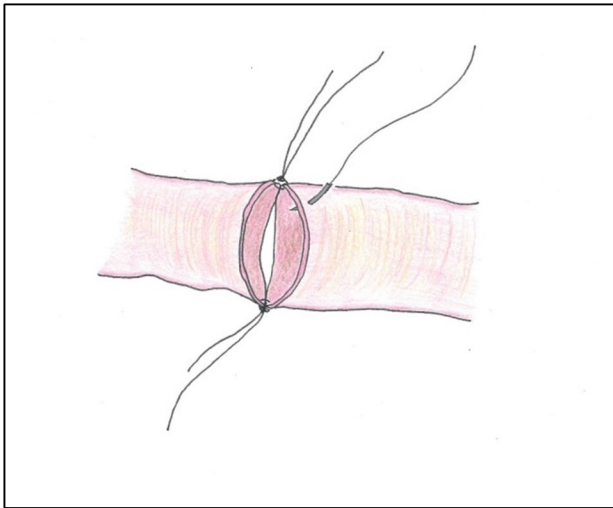
Conventional Method



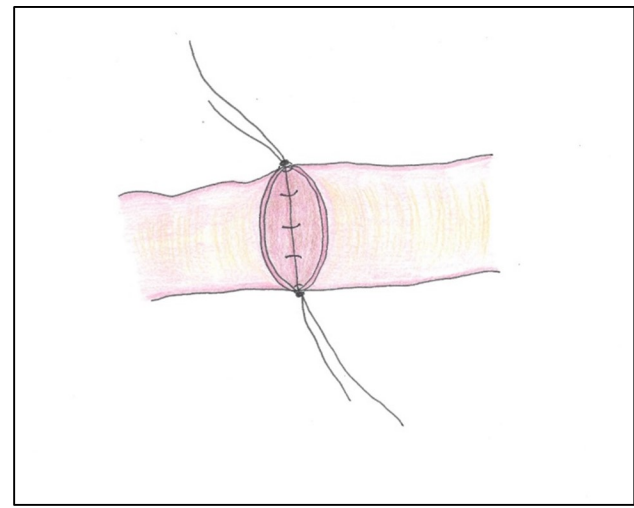
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b



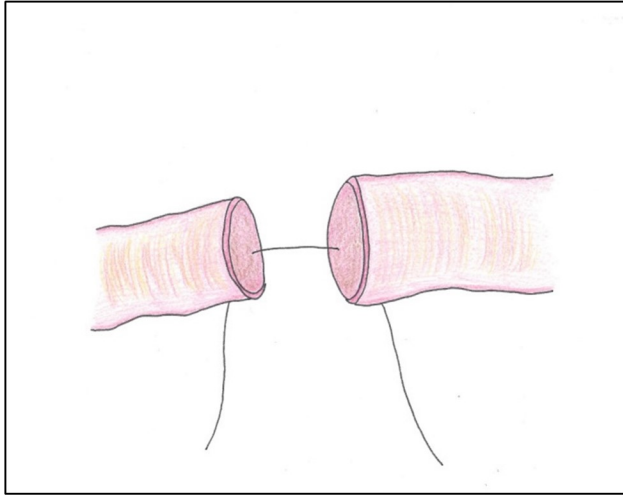
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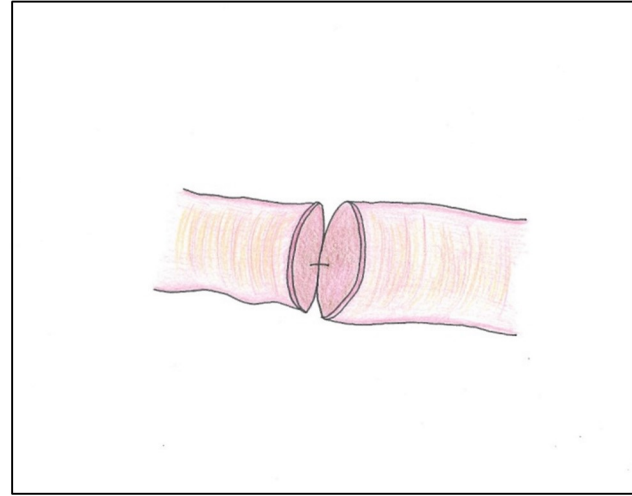
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Figure 1

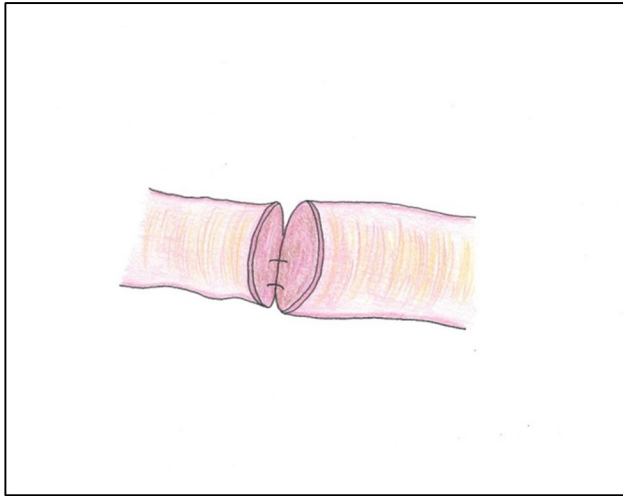
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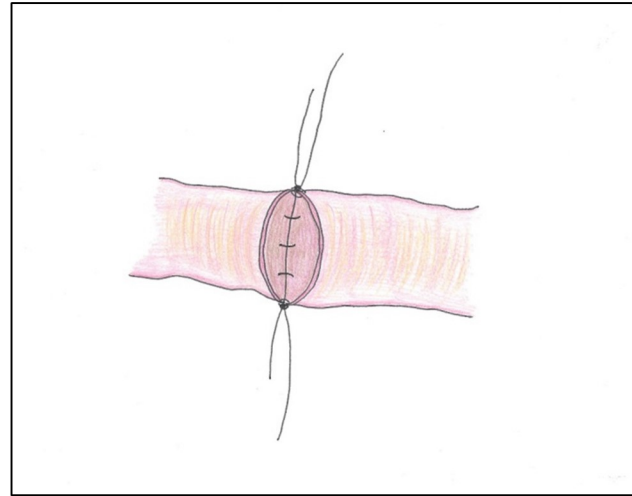
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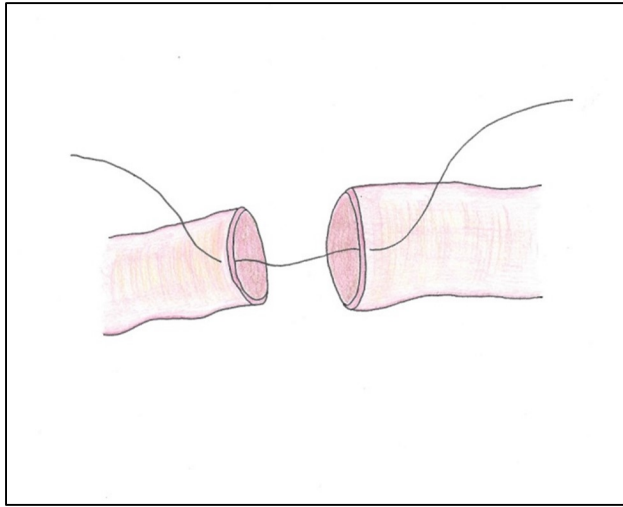
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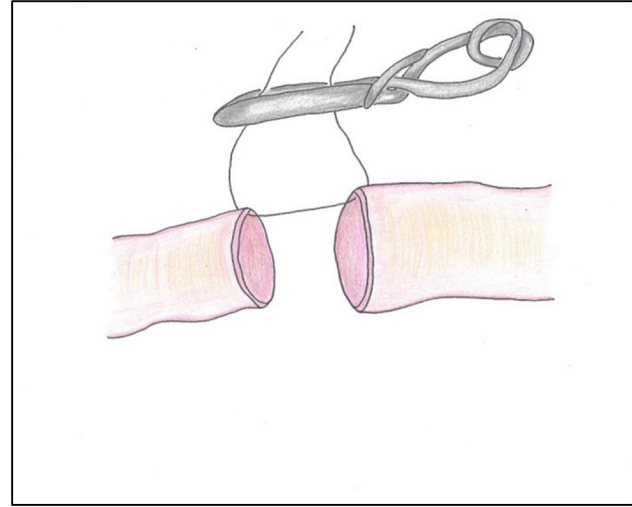
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Figure 2

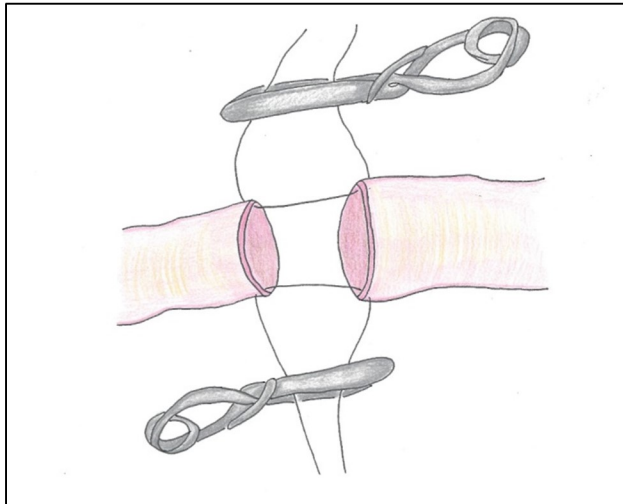
Untied Stay Suture Method



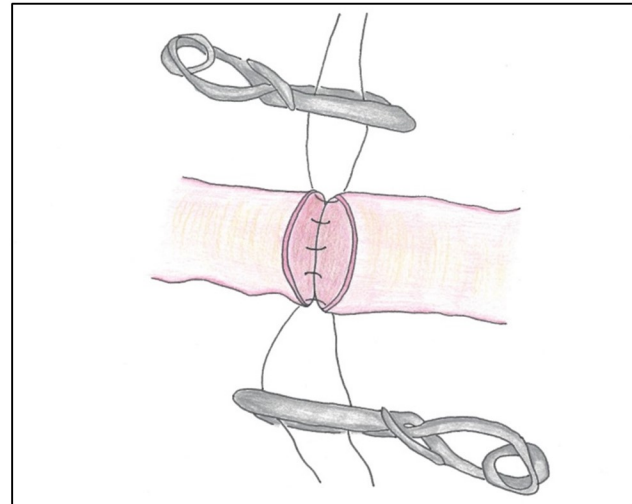
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b

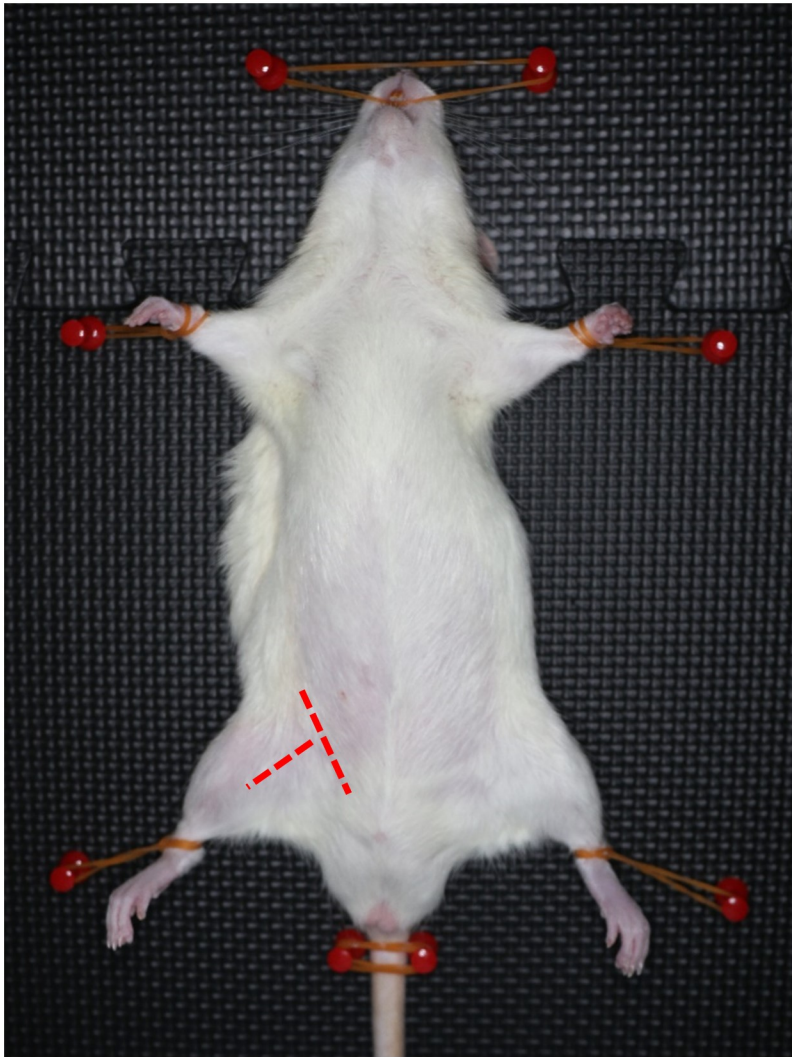


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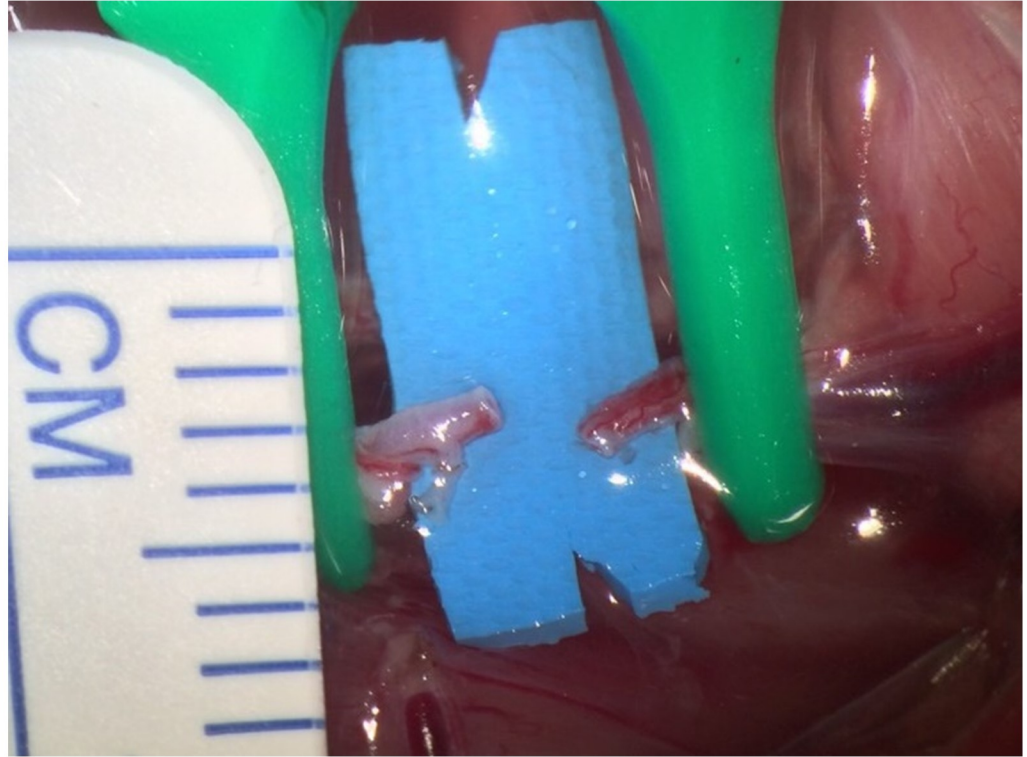


d

Figure 3



a



b

Figure 4