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## A Training and Testing System for Performing Vascular Reconstruction In Vitro --Manuscript Draft--

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**RE: Revised JoVE60141 “A Training and Testing System for Performing Vascular Reconstruction In Vitro” by Wang, Mu, Zhang, Chen, Li, Shi, Tang, Zhang, Dong and Lv.**

Dear Bajaj, PhD:

Thank you very much for your email dated September 19, 2019 in which you informed us that our manuscript has been editorially reviewed. We also appreciate for your excellent edit work and your critical comments. The comments, our point-by-point responses to them, and changes made in the text (which are highlighted by red font) are listed in separate file.

We sincerely hope that the changes made in the revised manuscript and video meet with your approval, and our manuscript and video can be published in the JoVE. If there are any further questions, please do not hesitate to contact us.

Sincerely yours,

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**KEYWORDS:**

vascular reconstruction, training system, testing system, magnetic tractor, magnetic suture puller, polypropylene suture damage

**SUMMARY:**

Here we present a training and testing system where a trainee can complete manual vascular reconstruction in vitro individually using a magnetic anchoring technique. The system can also be used to test the quality of reconstruction.

**ABSTRACT**

Manual vascular reconstruction training is essential for a beginner surgeon. However, an optimal training system for vascular reconstruction in vitro has yet to be developed. In this study, we introduce an in vitro training and testing system using a magnetic anchoring technique with which a trainee can practice manual vascular reconstruction individually. Additionally, this system can also be used to test the quality of the reconstruction. The described system includes a vascular reconstruction training machine, magnetic tractors, and

a magnetic suture puller. In this manuscript, we detail an end-to-end vein anastomosis using porcine right and left iliac veins. To identify the potential damage caused by a magnetic suture puller on the suture, we created three groups with six segments of 4-0 polypropylene sutures each: a control group with no intervention on the polypropylene suture, a group in which the polypropylene suture is manually pulled with sterile gloves 20x, and a magnetic puller group in which the magnetic puller pulled the polypropylene suture 20x. These groups were tested by light microscopy and breaking strength tests, and the effect of reconstruction was assessed. In the light microscopy test, the control group was less likely to be damaged ( $p < 0.05$ ) and the number of damaged points of the manual group and magnetic puller group were similar ( $p > 0.05$ ). The results of the breaking strength test were compared across groups and no significant difference was observed ( $p > 0.05$ ). The end-to-end anastomosis of the porcine iliac veins was successfully performed using this training system, and the reconstructed veins could undergo 2.0 kPa perfusion pressure. Using this training and testing system the trainee can practice manual vascular reconstruction in vitro individually with the aid of magnetic tractors and a magnetic suture puller, and the quality of the reconstruction can be tested.

## INTRODUCTION:

Vascular reconstruction is a basic skill required for surgeons. Although Obora<sup>1</sup> and Holt<sup>2</sup> invented several mechanical reconstruction methods to simplify the reconstruction of small vessels (diameters  $<10$  mm), these methods are not commonly applied in macrovascular anastomosis. Manual vascular anastomosis is still performed in many operations, including vascular surgery<sup>3</sup>, emergency surgery<sup>4</sup>, and solid organ transplantation<sup>5</sup>. Thus, it is essential for surgeons to practice manual vascular anastomosis. However, an optimal training system for vascular reconstruction in vitro is uncommon, and inexperienced surgeons must undergo considerable training in vivo on large animals<sup>6</sup> before they can master the technique. Because failure is inevitable during initial training, many animals are likely to die of vascular complications, which is concerning regarding animal welfare. Further, during the procedure of end-to-end vascular reconstruction, to avoid mistakes in stitch positions or loose sutures, the surgeon needs at least one assistant to expose the posterior vascular wall and pull the suture. Thus, vascular reconstruction usually cannot be performed by the surgeon individually, and the efficiency of preparation is usually limited by the proficiency of the assistant.

Magnetic anchoring surgery has become a topic of interest in recent years<sup>7-11</sup>. The clinical trial by Rivas et al.<sup>7</sup> showed that with his magnetic surgical instrument and following the principle of magnetic anchoring, surgeons can perform reduced-port laparoscopic cholecystectomy. The use of this instrument also allows for a reduced role for the assistant during open surgery. Through the magnetic field, the magnetic device is adsorbed onto an anchoring point. This magnetic anchoring device can act as a mechanical arm, grasping and retracting the tissue or organ, exposing the surgical field, and simplifying the operation. Based on this rationale, we invented magnetic tractors to retract the vascular wall and suture, and a magnetic suture puller to pull the polypropylene sutures.

The use of a vascular reconstruction training machine was another milestone in this study. It consists of an operating floor and a control panel: the vasculature is fixed on the operating

floor, and the trainee can practice on it. After anastomosis, the trainee can set the perfusion parameters on the control panel in order to test the quality of anastomosis. Compared to previous vascular anastomosis training systems<sup>6,12-14</sup>, the use of this system provides two main advantages: First, magnetic devices can be used to expose the surgical field, so that the trainees can practice on it individually. Second, the trainee can check the effect of anastomosis using a perfusion test.

In the present study, we introduce a training and testing system where the trainee can complete manual vascular reconstruction in vitro individually using a magnetic anchoring technique and the quality of reconstruction can also be tested. Limited by the design and size of the water inlet and water outlet on the operating floor, the training system can only perform end-to-end reconstruction on vessels with a >5 mm diameter.

## PROTOCOL:

The protocol was carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals and was approved by the Committee on the Ethics of Animal Experiments of Xi'an Jiaotong University, Xi'an, Shaanxi Province, China.

### 1. Preparation prior to the training

NOTE: The vascular reconstruction training machine is shown in **Figure 1**. It consists of a control panel and an operating floor.

1.1. Click the **Clean** button on the control panel to clean and drain the residual liquid from the operating floor.

1.2. Click the **Add Liquid** button on the control panel and add 0.9% saline into the machine from the operating floor until the prompt "**The testing liquid is adequate**" appears on the control panel.

1.3. Prepare the magnetic tractor, which consists of a circular permanent magnet with a diameter of 20 mm and a thickness of 1 mm, an acrylonitrile butadiene styrene (ABS) plastic casing, a spiral spring, a 30 cm nylon traction wire, and a stainless-steel clamp with plastic sleeves or a vascular clamp.

1.3.1. Glue the circular magnet and the plastic casing together using an acrylate adhesive. The traction force will increase with the lengthening of the traction wire. Use a universal testing machine to test the association between the length of the traction wire and the traction force (**Figure 2**).

1.3.2. Fix the clamp and the plastic casing on the upper holder and the lower holder of the universal testing machine, respectively. Gradually elevate the upper holder to stretch the traction wire between the two holders. Test the strength of the traction wire while it is being

133 stretched.

134  
135 NOTE: The magnetic suture tractor and magnetic vascular tractor are shown in **Figure 3**.

136  
137 1.4. Prepare a magnetic suture puller.

138  
139 1.4.1. Use a quasi-oval polylactic acid board with a thickness of 2 mm, a major axis diameter  
140 of 10 cm, a minor axis diameter of 2 cm, three magnetic balls with a diameter of 5 mm, and  
141 three magnetic cylinders with a diameter of 5 mm and a height of 5 mm.

142  
143 1.4.2. Punch three holes with a diameter of 3 mm and a depth of 0.5 mm on the polylactic  
144 acid board, so that the magnetic balls can cling to the board by magnetic attraction force from  
145 the magnetic cylinders under the board.

146  
147 NOTE: The magnetic suture puller is shown in **Figure 4**.

148  
149 1.5. Fix the suture under the magnetic ball after one stitch. This plays the role of a suture  
150 puller, preventing the previous suture from loosening. Extract the end with the suture needle  
151 with a force of about 0.3 N, closely in parallel to the polylactic acid board, and continue the  
152 next stitch.

153  
154 1.6. Ligate all the vein branches using 3-0 silk sutures to avoid leakage after anastomosis. Use  
155 tissue scissors to trim the ends of the veins and clear the excess tissue on the wall of the veins  
156 to make the veins smooth.

157  
158 NOTE: The vasculature used in this study included the right and left iliac veins (diameter ~10  
159 mm) harvested from Bama pigs weighing 50–60 kg. To simplify the reconstruction, only a few  
160 branches of the iliac veins were picked, and the two veins were similar in size. The vasculature  
161 was kept at -20 °C. Before training, it was immersed in 0.9% saline at room temperature.

## 162 163 **2. Fix the veins on the operating floor**

164  
165 2.1. Tie the two veins on the water inlet and water outlet of the training machine with 2-0 silk  
166 sutures.

167  
168 NOTE: This study uses the two-point vascular anastomosis<sup>5</sup>.

169  
170 2.2. Adjust the length of the water outlet of the training machine and ensure that the ends of  
171 the two veins are tension-free in a parallel direction.

172  
173 2.3 Straighten the veins and lay two 4-0 polypropylene traction sutures at the 6 o'clock and  
174 12 o'clock positions.

175  
176 2.4. Insert the needle of the traction sutures from the outside of the vein and then insert from

the inside of the other vein.

2.4. Wet the surgical glove and sutures to avoid damaging the sutures. Gently tie at least five knots to avoid tearing the walls of the veins.

2.4. Use the two stainless-steel clamps of the magnetic suture tractor to grasp the traction sutures and attract the circular magnets of the magnetic suture tractors to the ferromagnetic stainless-steel operating floor. Adjust the position of magnetic attraction and ensure that the ends of the two veins are stretched in a vertical direction.

2.5. Use the two vascular clamps of the magnetic vascular tractor to clamp the anterior wall of the veins and attract the circular magnets of the magnetic vascular tractors on the operating floor. Adjust the position of attraction and ensure that the anterior walls of the veins are retracted, and the posterior walls of the veins are clearly exposed.

### **3. Anastomosis of posterior walls**

3.1 Use the two stainless-steel clamps of the magnetic suture tractor to grasp the traction sutures and attract the circular magnets of the magnetic suture tractors on the ferromagnetic stainless-steel operating floor. Leave the tail segment of the polypropylene suture at the 12 o'clock position for the traction suture and use the segment with the needle for continuous suture.

3.2 Ensure intima-to-intima contact between the two veins.

3.3. Insert the first suture from the outside of the vein to the inside.

3.4. In subsequent sutures, insert the needle from the inside of the vein and then insert from the outside of the other vein.

3.5. Check that the sutures are not loose.

3.6. After one suture, ensure that the polypropylene suture is hung on the magnetic suture puller and pull the polypropylene gently until the magnetic ball presses the polypropylene.

3.7. Extract the end with the needle of the suture with a force of about 0.3 N, closely in parallel to the polylactic acid board, and continue the next stitch.

NOTE: By using this technique, the tail of the polypropylene suture will be sufficiently tight. As the sutures continue, the polypropylene suture will become shorter. According to the length of the suture, select the most suitable of the three magnetic balls, and then manually press the suture under it.

3.8. Insert the last suture from the inside of the vein to the outside to ensure intima-to-intima

contact between the two veins.

3.9. Avoid stenosis after anastomosis by two means: Keep same, proper margin and needle spacing when stitching, and keep the "growth factor"<sup>15</sup> when knotting.

NOTE: The "growth factor" is the reserved space away from the vessel wall when tying the first knot after the anastomosis so that vessels can remain flexible rather than stenose.

3.9.1 Maintain the same suture margin and needle spacing.

NOTE: In this study, iliac veins with a diameter of approximately 10 mm were used, so the suture margin and needle spacing was about 1 mm.

3.9.2 Keep the "growth factor"<sup>15</sup> when tying the knots. After anastomosis of the posterior walls, tie the end of the suture and the tail segment of the suture at the 6 o'clock position together away from the vein wall in order to prevent suture stenosis. Use the standard method for tying knots.

#### **4. Anastomosis of anterior walls**

4.1 After anastomosis of the posterior walls, remove the magnetic vascular tractor, leave the tail as a traction suture, and use the segment with the needle at the 6 o'clock position for the anastomosis of the anterior walls.

4.2 Insert the needle from the outside of the vein and then insert from the inside of the other vein.

NOTE: The methods used in the anastomosis of the posterior walls to ensure the intima-to-intima contact between the two veins (the suture not being loose and avoiding stenosis after anastomosis) were followed in the anastomosis of the anterior walls<sup>5,15</sup>.

4.3 After anastomosis of the anterior walls, cut off two traction sutures using suture scissors.

#### **5. Test the effect of anastomosis**

5.1 Set the test parameters.

5.1.1 Set the perfusion pressure as 2.0 kPa on the control panel.

NOTE: The normal vein pressure will not exceed 2.0 kPa.

5.1.2 Set the duration of peak pressure as 5 s on the control panel.

5.1.3 Set the temperature as 25 °C on the control panel.



5.1.4 Set the pressure deviation as 0.1 kPa on the control panel.

5.2 Click the **Test** button and observe the time and pressure on the control panel and whether the reconstructed vein leaks.

NOTE: If the vein does not leak during the peak pressure, the anastomosis is successful. If leaks are found, the leakage position should be located and sutured, and then the test should be performed again. The results of the test in this video are shown in **Figure 5**.

## 6. Checking the safety of the magnetic suture puller

NOTE: To test whether the magnetic suture puller damaged the polypropylene suture, perform the breaking strength and light microscopy tests. In this experiment, three groups with six segments of 4-0 polypropylene suture in each were tested: a control group with no intervention on the polypropylene suture, a manual group in which the polypropylene suture was manually pulled with sterile gloves 20x, and a magnetic puller group in which the magnetic puller pulled the polypropylene suture 20x.

6.1 Test the breaking strength of the polypropylene suture on the universal testing machine. Fix the two ends of the polypropylene suture on the upper holder and the lower holder of the universal testing machine. Gradually elevate the upper holder. Test the strength of the polypropylene suture while it is being stretched. Set the breaking strength as the tension force when the suture snaps. Compare the breaking strength among the three groups and perform pairwise comparisons.

6.2 Observe the damage of the polypropylene suture under a light microscope. Define the number of damage points as the number of fibrous or coarse fracture points visible at 200x magnification. Compare the number of damage points among the three groups and perform pairwise comparisons.

## REPRESENTATIVE RESULTS

The vascular reconstruction training machine is shown in **Figure 1** and includes two main parts: the operating floor and the control panel. The operating floor consists of a water inlet, a water outlet, and a water storage basin. The two ends of the vasculature are tied to the water inlet and water outlet to test the effect of anastomosis. The length of the water outlet is adjustable, and we set the parameters (e.g., the perfusion pressure, duration of peak pressure, temperature, and pressure deviation) on the control panel. In addition, we can observe the pressure curve on the control panel when the vasculature is tested.

The magnetic suture tractor and the magnetic vascular tractor are shown in **Figure 3**. The length of the traction wire is 30 cm, and the traction force increases with the lengthening of the traction wire (**Figure 2**). The range of the magnetic tractor's traction force is 0–1.8 N, which covers the range of traction force required for suture and vascular traction.

Photos of the magnetic suture puller are shown in **Figure 4A,B**. The three magnetic balls have a diameter of 5 mm, and the magnetic cylinders have a diameter of 5 mm and a height of 5 mm. These can be replaced by smaller or bigger ones. The suture pulling force will change accordingly.

In testing the effect of anastomosis, a time-perfusion pressure curve was generated and is shown in **Figure 5**. The perfusion pressure ascended to 2.0 kPa, which we set as the peak pressure. This was maintained for 5 s, which was set as the duration of peak pressure.

Regarding the safety of the magnetic suture puller, we tested whether the magnetic suture puller damaged the polypropylene suture using a breaking strength test and a light microscope. As shown in **Figure 6**, the breaking strength test results of the three groups were compared pairwise, and no significant difference was observed ( $p > 0.05$ ). As shown in **Figure 7**, the control group was less likely to be damaged ( $p < 0.05$ ), but the number of damaged points in the manual group and the magnetic puller group were similar ( $p > 0.05$ ).

#### FIGURE LEGENDS

**Figure 1: The vascular reconstruction training machine's two main parts.** The operating floor and the control panel.

**Figure 2: The association between the length of the traction wire and the traction force.** The length of the traction wire was 30 cm, and the range of traction force that the magnetic tractor could provide was 0–1.8 N.

**Figure 3: The magnetic suture tractor and the magnetic vascular tractor. (A)** Magnetic suture tractor. **(B)** Magnetic vascular tractor.

**Figure 4: The magnetic suture puller. (A)** Front view. **(B).** Lateral view. The magnetic suture puller consists of a quasi-oval polylactic acid board with a thickness of 2 mm, a major axis diameter of 10 cm, a minor axis diameter of 2 cm, three magnetic balls with diameter of 5 mm, and three magnetic cylinders with a diameter of 5 mm and a height of 5 mm.

**Figure 5: Time-perfusion pressure curve.** The perfusion pressure ascended to 2.0 kPa, which we set as the peak pressure. It was maintained for 5 s, indicating that the anastomosis was successful.

**Figure 6: The breaking strength test. (A)** The association between the length of the polypropylene suture and the tension. **(B).** Comparison of the breaking strength among the three groups. There was no significant difference in the three groups ( $p > 0.05$ ).

**Figure 7: The light microscope tests. (A)** Control group. **(B)** Manual group. **(C)** Magnetic puller group. **(D)** Comparison of the number of damage points among the three groups. The control group had fewer damage points ( $p < 0.05$ ), but there was no significant difference between

the manual group and the magnetic puller group ( $p > 0.05$ ). The black arrow points to the damage point. The asterisk represents the significant difference.

## **DISCUSSION:**

With the help of magnetic tractors and a magnetic suture puller, a trainee can complete vein anastomosis individually and precisely. Magnetic tractors pull the tissue that blocks the anastomosis field and provide suitable strength for stretching the veins in a vertical direction, thus achieving clear exposure for vein anastomosis. In traditional manual anastomosis, at least one assistant is required for surgical exposure. The use of magnetic tractors could achieve the required exposure and substitute for assistants. In addition, the traction force of the magnetic tractor was dependent on the length of the traction wire, so we could adjust the site that the magnetic tractor was adsorbed onto in order to change the length of the traction wire to obtain a suitable traction force. In contrast to traditional manual anastomosis, the traction force in this study was quantifiable by the length of the traction wire. This allowed us to avoid some issues resulting from too heavy or too light a traction force, such as tearing of the vasculature and unclear exposure.

The magnetic suture puller was another novel invention in this study. It replaced the requirement for an assistant to pull the suture to prevent the previous suture from loosening, resulting in anastomotic leakage. Because it pressed the polypropylene suture, we tested the degree of damage caused by the magnetic suture puller and compared it to intact and manual pulling. Although the number of damage points in the magnetic puller group was more than in the control group (intact polypropylene sutures), it was similar to that observed in the manual pull that is widely used in clinical practice. Moreover, the breaking strength test showed a similar breaking strength among the three groups. With the microscope, we found that the changes caused by the magnetic puller were too small to damage the strength of the polypropylene suture.

It must be emphasized that the tension on the vertical and parallel directions of the vasculature during anastomosis is significant. Therefore, it is vital to adjust the length of the water outlet of the training machine as well as the position of the magnetic tractors. In addition, as we add sutures, we pick the most suitable magnetic ball to press the suture so that the tension on the suture is moderate. Moreover, to avoid stenosis after anastomosis, it is essential to keep the same suture margin, needle spacing, and "growth factor".

If the trainee wishes to practice anastomosis using vasculature with a greater or smaller diameter, the magnetic balls and cylinders of the magnetic suture puller should be replaced by bigger or smaller ones, so that the pulling force changes accordingly. Simultaneously, the test parameters after anastomosis should be adjusted. In the current version of the vascular reconstruction training machine, the diameter of the inlet and outlet is only 5 mm, making it difficult to use on smaller diameter vessels. Fortunately, the inlet and outlets are detachable, so the current inlet and outlet can be replaced by smaller ones that allow changes in vessel size.

Beside the sizes of the inlet and outlet, there are still some limitations to this training system. Because there is only one water inlet and one water outlet, this training and testing system is only applicable to end-to-end anastomosis, and trainees cannot practice end-to-side or side-to-side anastomosis using this system. Additionally, the surgical instruments used in this video (e.g., the needle holder and scissors) are ferromagnetic stainless steel. They were absorbed by the magnetic tools occasionally, which could interfere with the training progress. If conditions permit, the surgical instruments can be replaced with non-ferromagnetic titanium instruments.

Open surgical vascular simulators are generally divided into two types: in vivo and in vitro. Tang<sup>6</sup> developed a novel technique for vascular reconstruction in vivo using ewes as animal models. Although this technique provided a more realistic operation scene, the use of in vivo animal models is both inconvenient for training and costly. Shimizu<sup>12</sup> and Maluf<sup>13</sup> invented in vitro training devices for cerebrovascular anastomosis, while Bismuth<sup>14</sup> introduced a vascular surgery course named Cardiovascular Fellows Bootcamp for cardiovascular surgery education. Although the rationale of our training system is similar to those outlined in these studies, no previous study has recommended the use of a device to assist in exposing surgical field and maintaining the tension of the suture. Thus, the training previously described must be completed by at least two trainees. Also, previous researchers did not introduce a way to precisely check the quality of anastomosis. Therefore, compared to these open vascular simulators, our technique is economical, convenient to practice individually, and effective in terms of feedback training quality.

We intend to add smaller water inlets and water outlets to the vascular reconstruction training instrument so that trainees can practice other types of anastomosis. We expect that magnetic tractors and suture pullers will be used to help surgeons expose the surgical field in routine clinical operations in the future.

In summary, we introduce a training and testing system where the trainee can complete manual vascular reconstruction in vitro individually with the aid of magnetic tractors and a magnetic suture puller.

#### **DISCLOSURES:**

The authors have nothing to disclose.

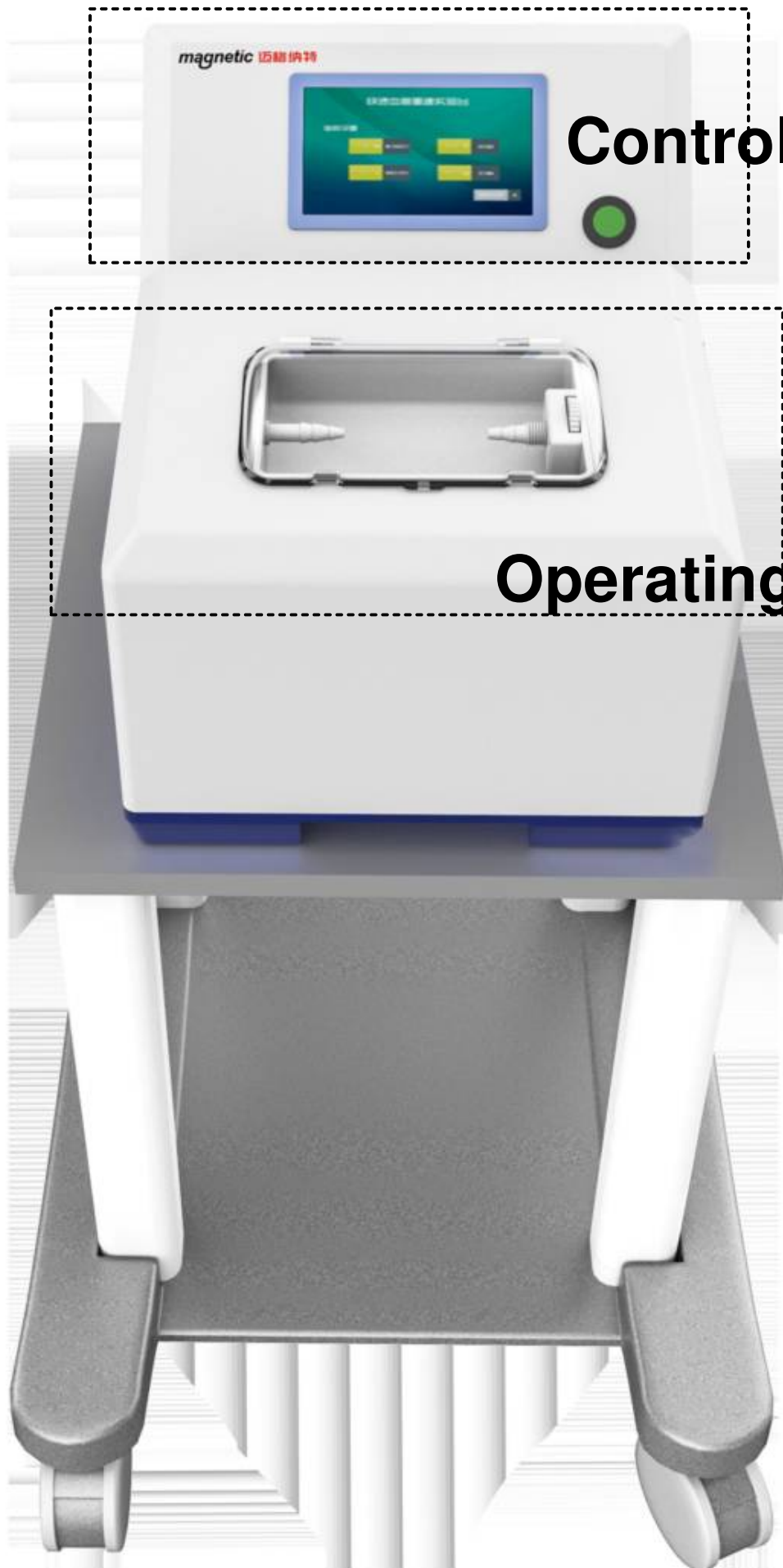
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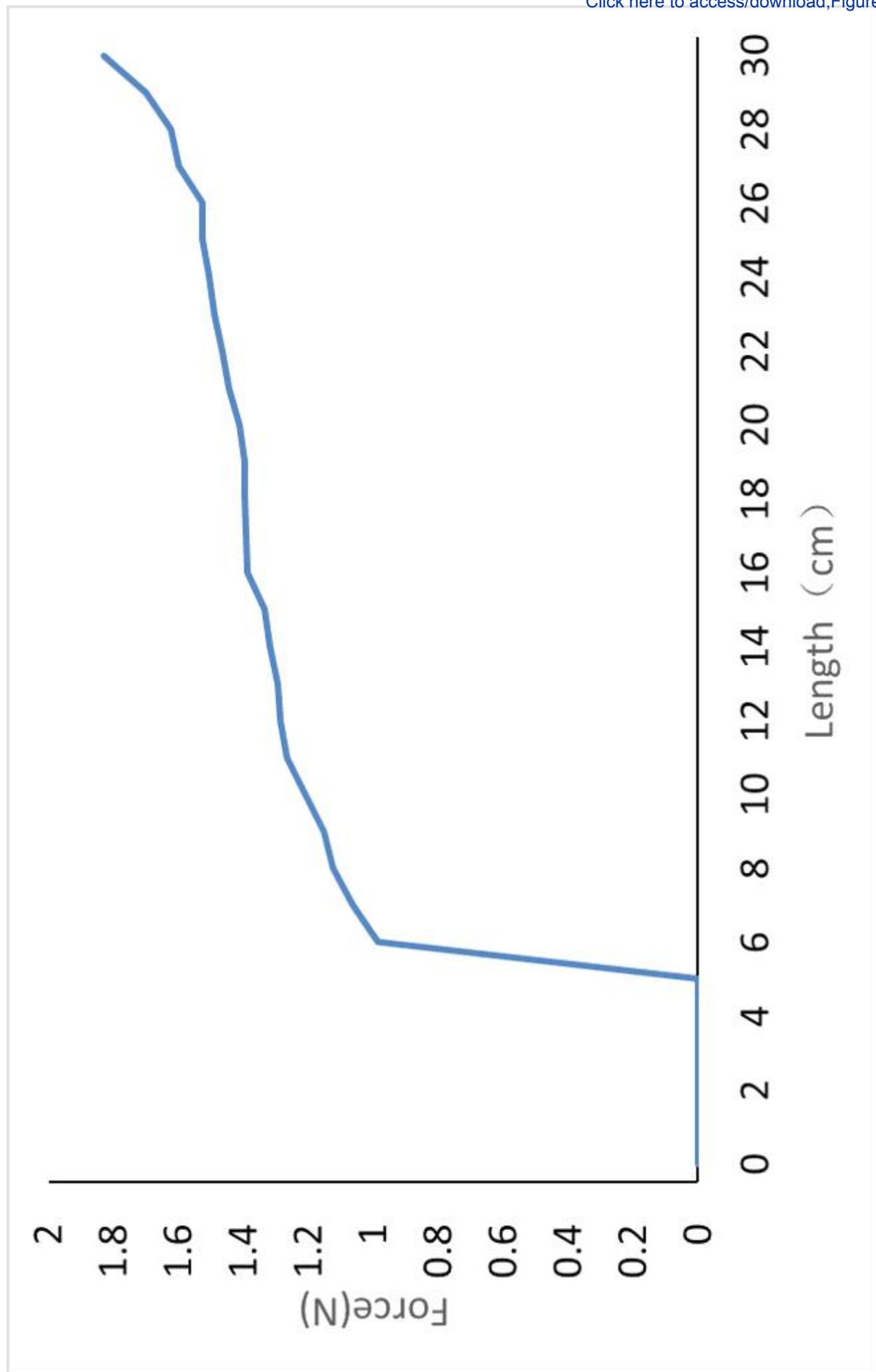
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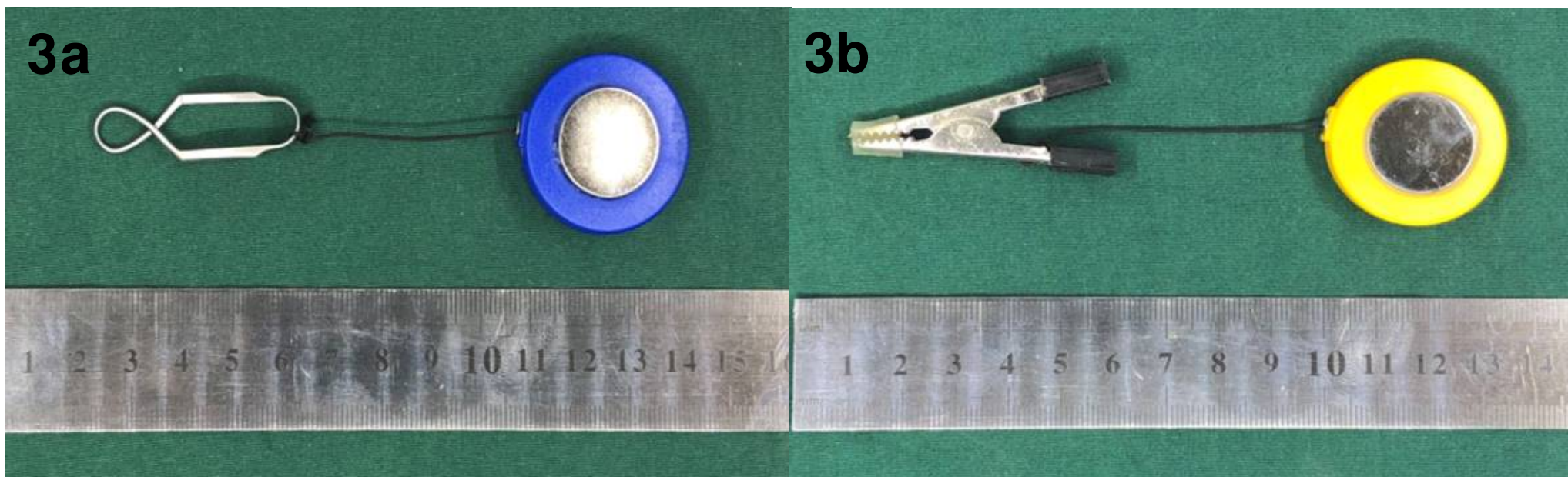
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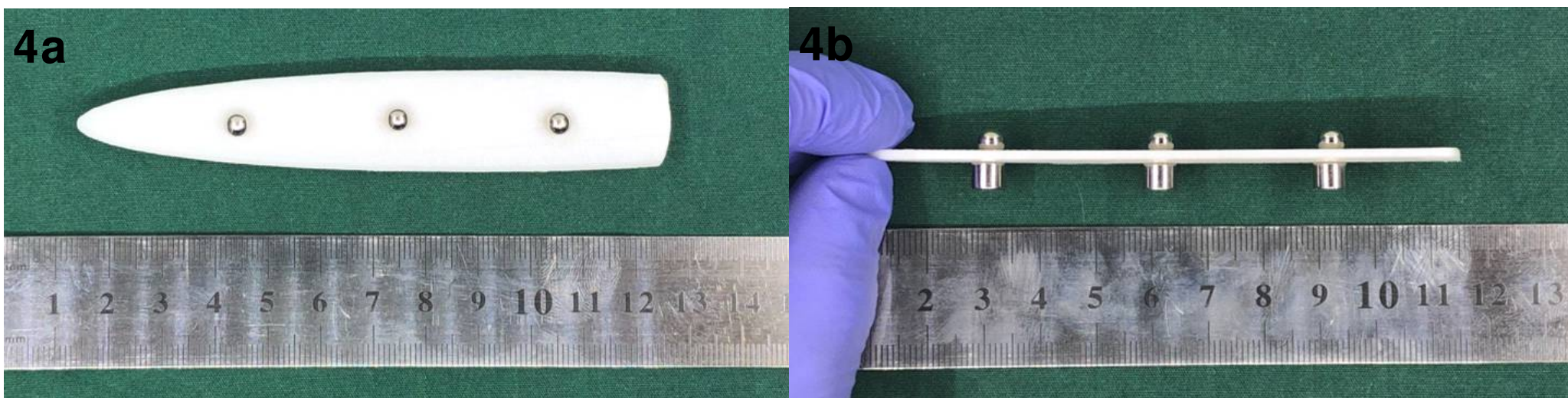
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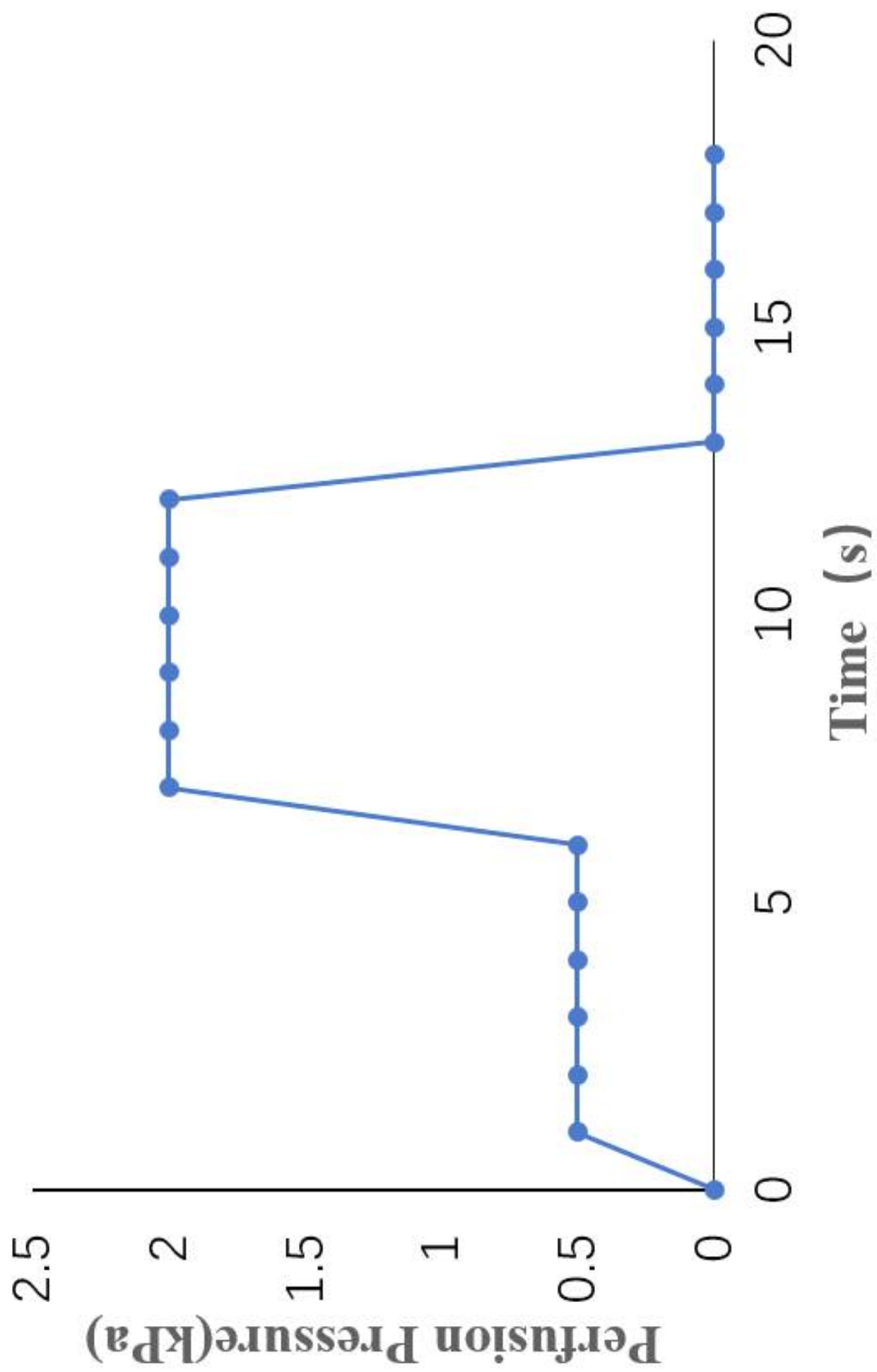




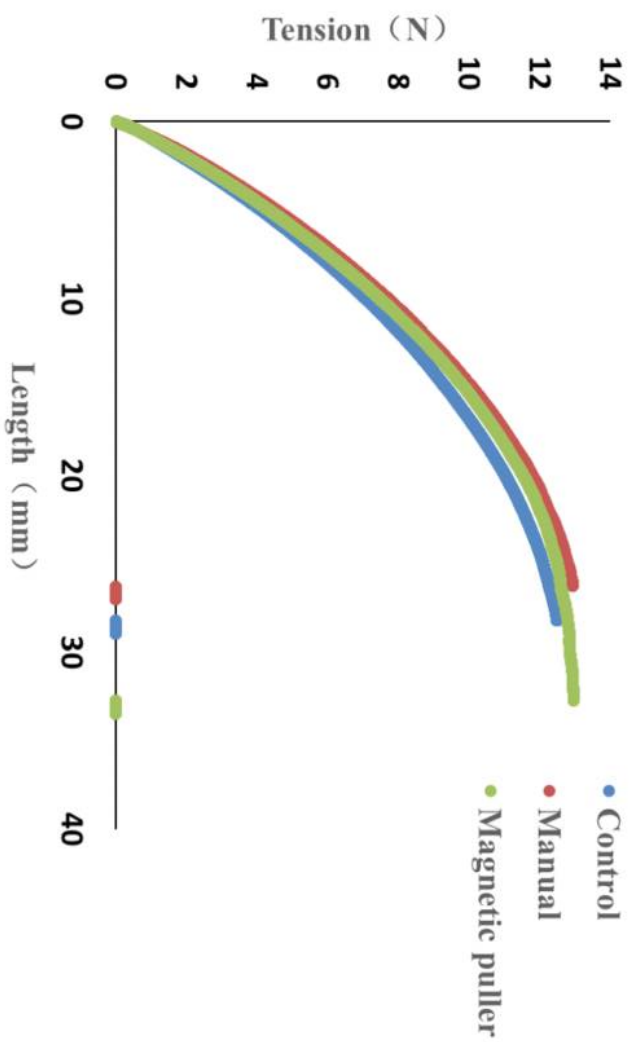




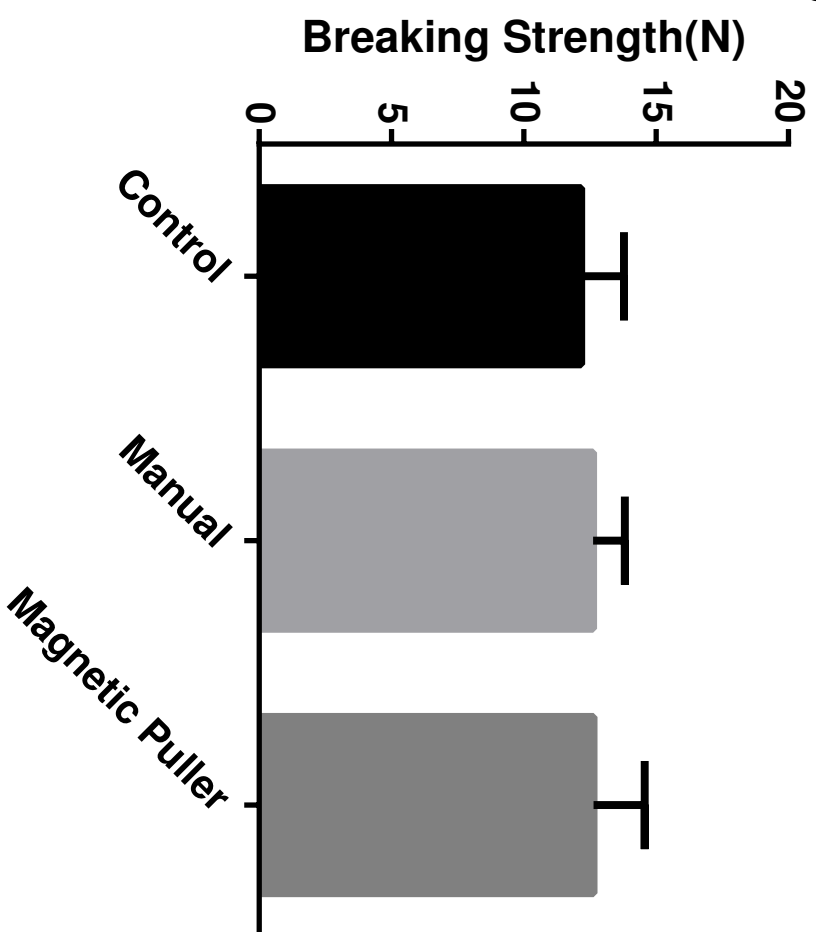


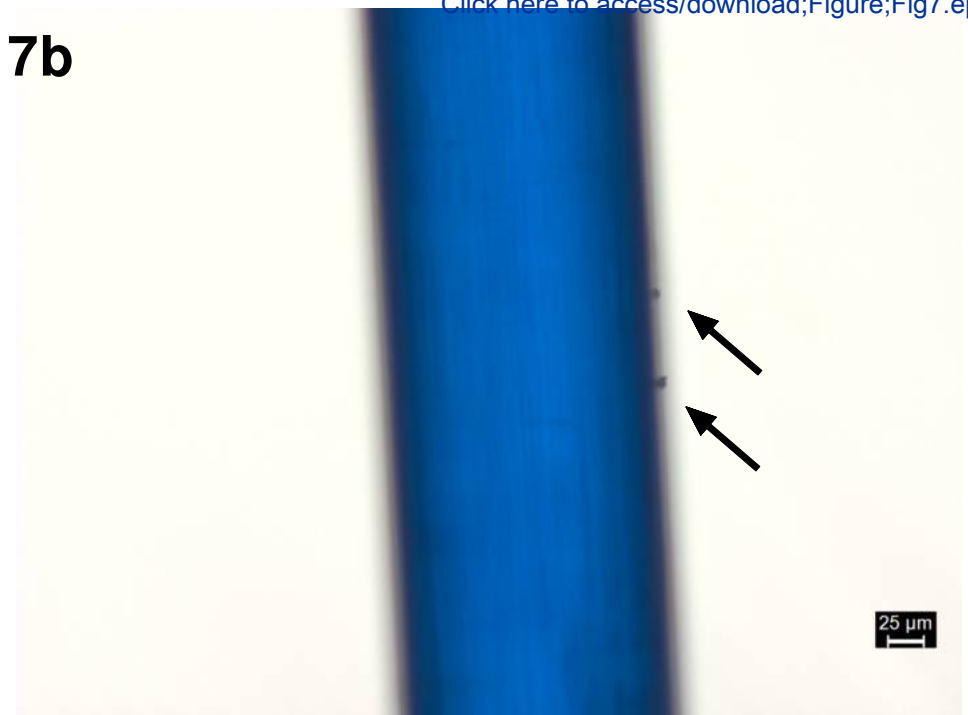
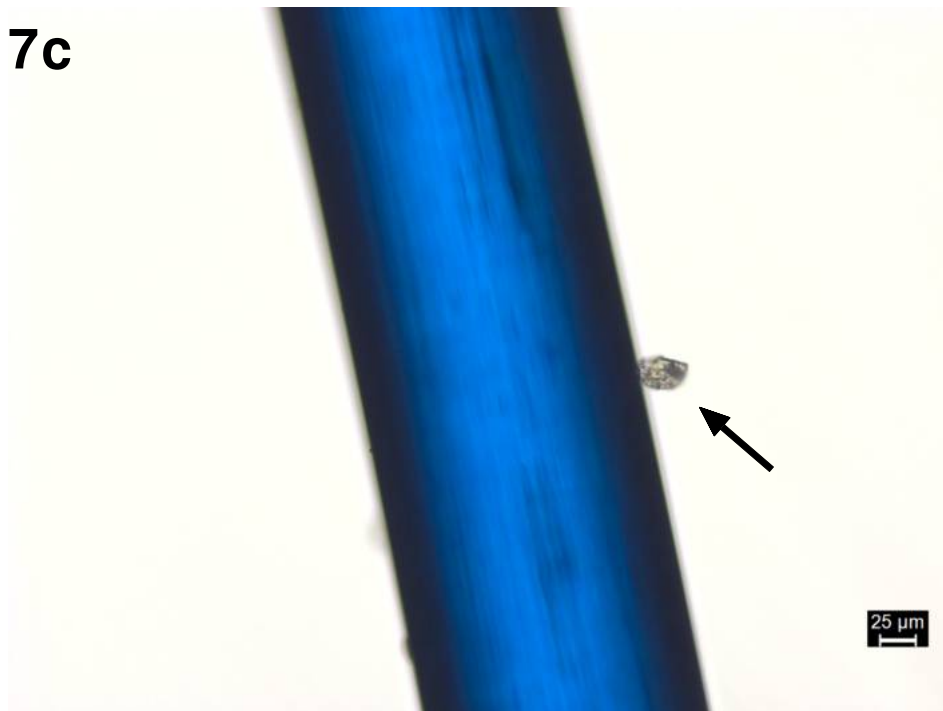
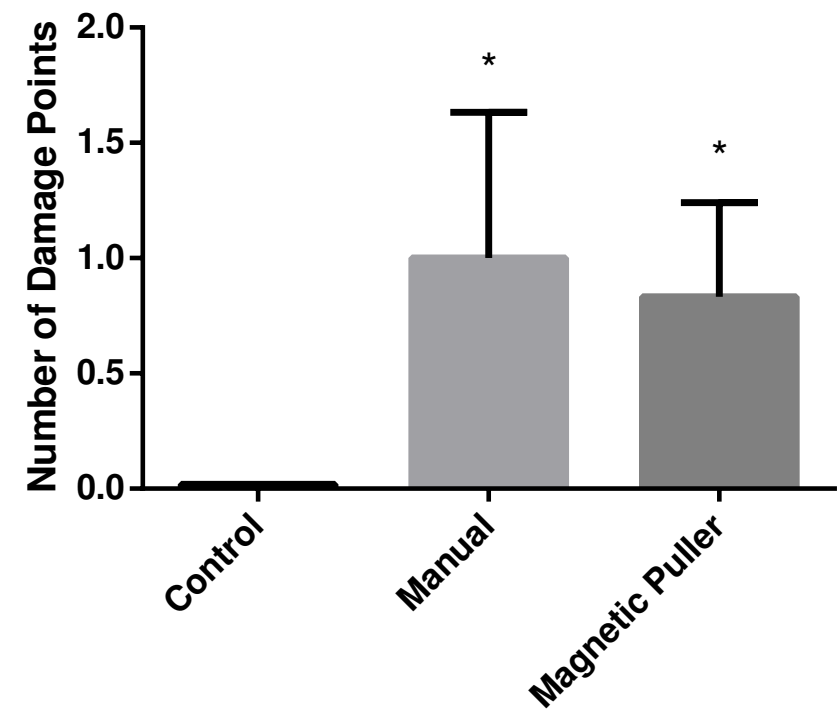


6a



6b



**7a****7b****7c****7d**

Name of Material/Equipment	Company	Catalog Number
Circular permanent magnet	Hangzhou Permanent Magnet Group Co.LTD	20*1mm
Magnetic balls	Hangzhou Permanent Magnet Group Co.LTD	5mm
Magnetic cylinders	Hangzhou Permanent Magnet Group Co.LTD	5*5mm
Polypropylene suture	Johnson and Johnson	PROLENE 4-0
Silk suture	SILK	2-0, 3-0
Surgical insturments	Jinzhong Shanghai	JZ-2018
Universal testing machine	Zwick GmbH&Co	Z010

### Comments/Description

Magnetic tractor

Magnetic suture puller

Magnetic suture puller

Used for anastomosis

Used for fixing vascular and ligation

Suture scissors, tissue scissors, forceps, needle and needle holder

Used for testing the association between the length of traction wire and the traction force

## ***Responses to Editorial comments***

### ***Editorial comments:***

***1. The editor has formatted the manuscript to match the journal's style. Please retain the same.***

We appreciate for your excellent work. We retain this format in the revision.

***2. Please address specific comments marked in the manuscript text.***

According to comments, the changes (which are highlighted by red font in the manuscript) are made in the manuscript text (Lines 30-31, 127-130, 140-141, 147-149, 178, 198, 212-213, 216-218, 220-224, 231, 244-245, and 281-283).

And we address some specific comments in the comment text (Lines 84, 108, 140, 147-148, 161, 190, 217, 231, and 236).

***3. Please proofread the manuscript well before submission.***

Thanks for your suggestion. We have proofread the manuscript text carefully.

### ***Video:***

***The narration, while being consistently understandable, sounds very compressed. It is also low in volume (It should be peaking between -6 and -12 dB) and will need to be raised. Raising the volume will only amplify the issues with the audio quality. We would recommend re-recording with a better microphone.***

We thank you for pointing out this important issue. We re-recorded the audio using a better microphone.

***0:16 and 9:58 interviews - The audio is very low on these interviews. Like the narration, it should be peaking between -6 and -12 dB. But also, simply raising the audio will only amplify issues with the audio quality. We recommend reshooting the interviews, if possible, and either using a body microphone or placing the camera closer to the subject.***

We thank you for pointing out this important issue. We re-shot the interviews using a better microphone.

***We recommend sequential whole numbering for section title cards, rather than having Step 2 (1:28), Step 3 (4:34) and Step 4 (7:34) followed by 3. Representative Results (9:11). This will better correspond with our website chaptering system when the video is published.***

We apologize for making these mistakes. We delete the numbering of the subheadings in the revised video.





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Author(s):

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Yue Wang, Lijun Mu, Wei Zhang, Huanan Chen, Qingshan Li, Aihua Shi, Bo Tang, Xiaogang Zhang, Dinghui Dong, Yi Lv

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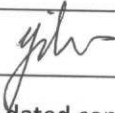
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