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Detailed setup methodology of a new ex vivo model for evaluation of endoscopic submucosal injection materials performance

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TITLE:

A New *Ex Vivo* Model for the Evaluation of Endoscopic Submucosal Injection Material Performance

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KEYWORDS:

Endoscopic mucosal resection, endoscopic submucosal dissection, submucosal injection material, *ex vivo* model, gastrointestinal neoplasms, submucosal elevation height

SHORT ABSTRACT

Here, we presented the detailed set up of a new *ex vivo* model that applies constant tension to the porcine gastric specimen. This development made it possible to evaluate the performance of various SIMs accurately, using the height and duration of the submucosal elevation.

ABSTRACT:

Increasing the performance of submucosal injection materials (SIMs) is important for endoscopic therapy of early gastrointestinal cancer. It is essential to establish an *ex vivo* model that can evaluate SIM performance accurately, for developing high-performance SIMs. In our previous study, we developed a new *ex vivo* model that can be used to evaluate the performance of various SIMs in detail by applying constant tension to the specimen's ends. We also confirmed that the proposed new *ex vivo* model allows accurate submucosal elevation height (SEH) measurement under uniform conditions and detailed comparisons of the performances of various types of SIMs are presented. Here, we describe the new *ex vivo* model and explain the detailed setup methodology of this model. Since all parts of the new model were easy to obtain, the setup of the new model could be completed quickly. SEH of various SIMs could be measured more accurately by using the new model. The critical factor that determines SIM performance can be identified using

the new model. SIM development speed will drastically increase after the factor has been identified.

INTRODUCTION:

Both endoscopic submucosal dissection (ESD) and endoscopic mucosal resection (EMR) are currently common treatments for early-stage gastrointestinal cancer[1,2]. Injecting a submucosal injection material (SIM) into the submucosa is one of the most important steps for both the EMR and ESD procedures[2,3]. High submucosal elevation and maintenance of submucosal elevation are critical criteria for safely conducting EMR/ESD.

Although normal saline (NS) has been used as a SIM since the invention of endoscopic therapy[4,5], sodium hyaluronate (HA) was introduced as a treatment in recent years[6,7]. HA became widely used in endoscopic treatments as a superior SIM due to its high performance[8-11]. Currently, a performance comparison between the existing SIMs was conducted, and high-performance SIMs were developed to identify another superior SIM[5,12-18].

The *ex vivo* model using a porcine stomach specimen has been used to evaluate SIM performance, because the estimation of SIM performance in the human gastrointestinal tract is very difficult[19-22]. However, this conventional *ex vivo* model is extremely simple, and has the scope for improvement. Reproducing an environment closer to the human gastrointestinal mucosa will enable accurate evaluation of SIM performance.

In our previous study, we developed a new *ex vivo* model that can be used to evaluate the performance of various SIMs in detail by applying constant tension to the specimen's ends. Using this new *ex vivo* model, accurate SHE measurement under uniform conditions and a detailed comparison of the performances of various types of SIMs are presented[23].

In this study, we present a complete appearance of the new *ex vivo* model, and the detailed setup methodology of the new *ex vivo* model is explained in detail. The material used in this new *ex vivo* model is easily available and the model can be quickly set up. Descriptions of detailed setup methodology will contribute to the dissemination of the new model.

PROTOCOL:

The following protocol follows the animal care guidelines of the Kyoto Prefectural University of Medicine.

1. Preparation of Specimens Using a Porcine Stomach

Note: The first step is to prepare specimens to be used in the *ex vivo* model (**Figure 1**). The

thickness of the porcine gastric wall varies in different areas of the stomach. Use the upper third of the porcine stomach, which is relatively similar to the human stomach. Exclude inappropriate specimens where submucosal elevation is not found due to fibrosis.

1.1. Cut the gastric specimens into squares with approximate dimensions of 5 × 5 cm.

1.2. Store the gastric specimens immediately at a temperature of −30 °C.

1.3. Thaw frozen gastric specimens right before the measurement procedure to ensure uniform measurement conditions.

2. Detailed Setup Methodology of a New *Ex Vivo* Model

Note: The thawed specimen can be stretched out on a board in two different ways. In the conventional *ex vivo* model, fix the specimen with pins (**Figure 1A**) [19-22]. On the other hand, in the new *ex vivo* model, fix or stretch both ends of the specimen with clips to produce a constant tension (**Figure 1B, C**). All parts of the new model are easy to obtain, and the setup of the new model can be completed quickly (**Figure 2**). The procedure of the new model is as follows (**Figure 3**):

2.1. Connect the stainless-steel clip, the key wire and the S shaped hook (**Figure 3A**).

2.2. Connect the wire, the S shaped hook and the weight (**Figure 3A**).

2.3. Connect the hook to the other end of the wire. A traction device is completed in the above process (**Figure 3B**).

2.4. Fix the pulleys (**Figure 2b**) at both ends of the base (**Figure 3C**).

2.5. Place the rubber plate (5 x 5 cm) on the center of the base (**Figure 3C**).

2.6. Place the gastric specimen on the rubber plate and pinch the specimen ends with the clip of the traction device.

2.7. Hang the weight through the pulley (both side). Thereby, constant tension can be applied to the specimen (**Figure 4**).

2.8. Start the measurement of SHE after the setup of the new model is completely finished.

3. Evaluation of SIM Performance

Note: In this study, we used normal saline (NS) and 0.4% sodium hyaluronate (HA) as SIMs to be tested, and measure the SEH of the two SIMs. Three independent measurements are performed. The obtained data are expressed as the mean and standard deviation (S.D.). Statistical analysis was performed by using the statistical analysis software (GraphPad Prism 7). We analyzed continuous variables (SEH) with the Student's t-test, and the magnitudes with $p < 0.05$ were considered significant. The measurement of SEH is as follows (**Figure 5**).

3.1. Perform zero-point adjustment of the height gauge, based on the height of mucosa before a submucosal elevation procedure. In detail, perform zero-point adjustment by pushing the **PRESET** button after fixing the scriber at the height of the mucosal surface.

3.2. Inject 2.0 mL of each solution horizontally into the submucosa from the specimen margins using a 2.5-mL syringe and 23G needle, to perform a submucosal elevation procedure (**Figure 5A-C**).

3.3. Measure the SEH promptly using a digital height gage at 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 30, 45, and 60 min after the injection (**Figure 5D**). In detail, record the height displayed on the height gauge when fixing the scriber to the top of the submucosal elevation.

3.4. Perform three independent measurements and express the obtained results as the mean and standard deviation.

3.5. Analyze the obtained data using appropriate statistical software and evaluate the performance of SIMs.

Note: The performance can be compared between each SIM.

REPRESENTATIVE RESULTS:

SEH was measured over time in the new *ex vivo* model or conventional *ex vivo* model. The values of SEH (NS) measured using the conventional model [NS was injected into the submucosa of the specimen fixed with pins (0.0 N)] were 5.7 mm (0 min), 3.6 mm (5 min), 3.0 mm (10 min), and 2.2 mm (30 min). In this way, the values of SEH decreased with increasing post injection time. A similar analysis was performed using 0.4% HA instead of NS. The values of SEH (0.4% HA) were 6.5 mm (0 min), 5.2 mm (5 min), 4.8 mm (10 min), and 4.1 mm (30 min). The resulting SEHs of 0.4% HA were higher than those of NS regardless of the post injection time. The SEHs (NS and 0.4% HA) obtained using the conventional model (in the absence of the applied tension) exhibited relatively large variations (in other words, their standard deviations were high) (**Figure 6A**).

Next, the values of SEH (NS) measured using the conventional model [NS was injected into the submucosa of the specimen stretched at a constant tension (1.5 N)] were 4.8 mm (0 min), 3.0 mm (5 min), 2.4 mm (10 min), and 1.8 mm (30 min). When the tension was increased to 3.0 N under the same conditions, the values of SEH (NS) were 4.5 mm (0 min), 2.3 mm (5 min), 1.5 mm (10 min), and 1.3 mm (30 min). The SEH measured at various post injection times decreased with increasing tension. The SEHs obtained using the new model exhibited small variations (in other words, their standard deviations were low) (**Figure 6B, C**).

For evaluating the relationship between SEH and tension applied to the specimen, we compared SEH measured at different tensions (0.0-3.0 N). In the analysis with the new model, the SEH obtained at a tension of 3.0 N was significantly lower than the SEH obtained at a tension of 1.5 N (in all cases, the condition $p < 0.001$ was satisfied). In contrast, since the standard deviations of SEHs obtained using the conventional model (0.0 N) were high, there was no significant difference between SEHs obtained using the conventional model (0.0 N) and the new model (1.5 N) (**Figure 6D, E**).

FIGURE LEGENDS:

Figure 1. New *ex vivo* model and conventional *ex vivo* model. In the conventional *ex vivo* model, the porcine specimen was fixed with pins **(A)**. On the other hand, in the new *ex vivo* model, both ends of the specimen were stretched with clips to produce a constant tension **(B)**. This model can be tensioned uniformly by using a weight, and the tension can be arranged by changing the weight **(C)**. Each SIM was injected into the submucosa of the specimen, leading to submucosal elevation **(D)**. This figure has been modified from Hirose *et al.*[23].

Figure 2. All parts used for the new model. The new *ex vivo* model consists of parts that are easily available. All parts used for the new *ex vivo* model: **(a)** approximately 50-300 g of weights (the weight can be changed appropriately depending on the applied tension); **(b)** fixed type pulley with the pulley diameter of 25 mm; **(c)** stainless steel wire with a diameter of 0.45 mm; **(d)** stainless steel clip with the width of 147 mm; **(e)** stainless steel key wire with a length of 12 cm; **(f)** stainless steel S shaped hook; **(g)** lockable stainless steel S-shaped hook. This figure has been modified from Hirose *et al.*[23].

Figure 3. The detailed setup of the new *ex vivo* model. The new *ex vivo* model can be quickly set up. **(A)** Connect the stainless steel clip (**Figure 2d**, the key wire (**Figure 2e**) and the S shaped hook (**Figure 2g**). Next, connect the wire (**Figure 2c**), the S shaped hook (**Figure 2f**) and the weight (**Figure 2a**). **(B)** Finally, connect the hook (Figure 2g) to the other end of the wire (**Figure 2c**). A traction device is completed in the above process. **(C)** Fix the pulleys (**Figure 2b**) at both ends of the base [rectangular wooden base (45 x 60 cm)

for assembling the model]. Next, place the rubber plate (5 x 5 cm) on the center of the base.

Figure 4. The complete appearance of the new *ex vivo* model. Accurate measurement of SEH can be performed.

Figure 5. The measurement procedure using the new *ex vivo* model. To evaluate SIM performance, the magnitude of SEH was measured by a digital height gage **(A)**. Using a 2.5-mL syringe with a 23G needle, 2.0 mL of each SIM was injected into the submucosa from the specimen margins to create a submucosal elevation **(B, C)**. The digital height gage was used to measure of the height of the submucosal elevation (*i.e.*, the values of SEH) **(D)**.

Figure 6. Measurement of SEH using either the new or conventional model. After the injection of NS or 0.4% HA into the submucosa of the specimen fixed with pins (0.0 N) **(A)** or stretched at a constant tension (1.5 N or 3.0 N) **(B, C)**, SEH was measured using the height gage. Next, we compared the values of SEH measured at different tensions (0.0, 1.5, and 3.0 N) after the submucosal injection of NS **(D)** or 0.4% HA **(E)**. Data are expressed as mean ± S.D. of more than three independent experiments. This figure has been modified from Hirose *et al.*[23].

DISCUSSION:

The porcine stomach used for the new model should be stored in a freezer immediately after the resection, and be used within a few months after freezing, since the freshness of the swine stomach is essential for SEH measurement. We measured the SEH using both frozen and unfrozen gastric specimens, and confirmed that there was no difference in the result of SEH measurement.

The quality of gastric specimens is greatly influenced by the individual differences of porcine stomachs. Hence, it is recommended to exclude obviously thick specimens or specimens with many folds before the measurement. Furthermore, some specimens may be inappropriate for SEH measurement due to fibrosis. It is recommended to exclude the inappropriate specimens where submucosal elevation is not found due to fibrosis.

Since the digestive tract is expanded by endoscopic treatment, some tension is applied to the gastrointestinal mucosa. It was revealed that SIM performance (evaluated by measuring the values of SEH) decreased with the tension applied to the specimens increasing. Therefore, the tension was an important factor affecting the SIM performance (*i.e.*, the values of SEH)[23]. The application of the tension (1.5-3.0 N) can reproduce an environment closer to the human gastrointestinal mucosa. However, a limitation of this method is that the optimal tension may depend on the difference of the specimen used

for analysis.

In the conventional model, since the tension applied to each specimen varies depending on the degree of specimen fixation, the variations of measured SEH are large (which correspond to the high standard deviations of SEH). Therefore, these high standard deviations make it difficult to compare each SEH in detail and perform statistical analysis. On the other hand, owing to small variations of SEH measured in the new model, SIM performance can be compared accurately *ex vivo* and precise statistical analysis is performed.

In conclusion, the new *ex vivo* model enables accurate SEH measurement and detailed comparison of SIM performance. Descriptions of detailed setup methodology will contribute to the dissemination of the new model and the development of high-performance materials.

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DISCLOSURES:
The authors have nothing to disclose.

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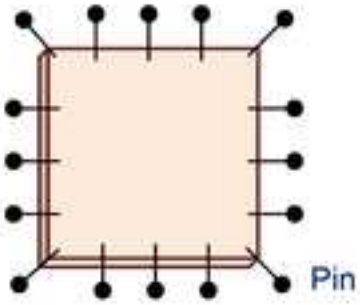
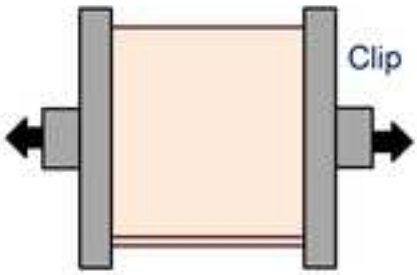
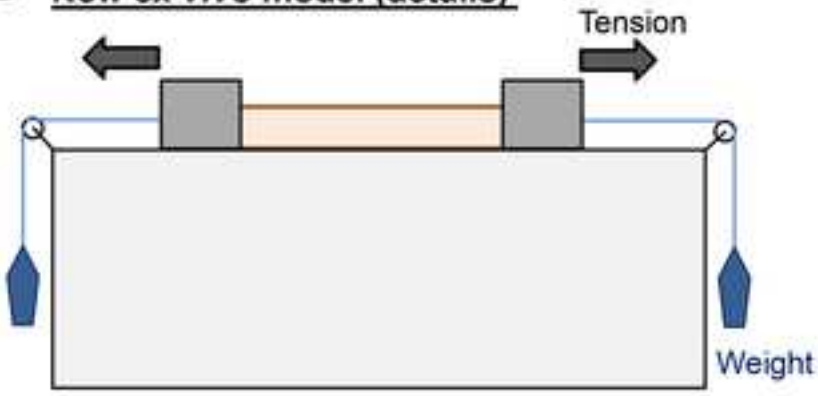
A **Conventional *ex vivo* model**



B **New *ex vivo* model**



C **New *ex vivo* model (details)**



Relationship between tension and weight

Tension (N)	Weight (g)
0.5	51.0
1.0	102.0
1.5	153.1
3.0	306.1

D **Submucosal elevation**

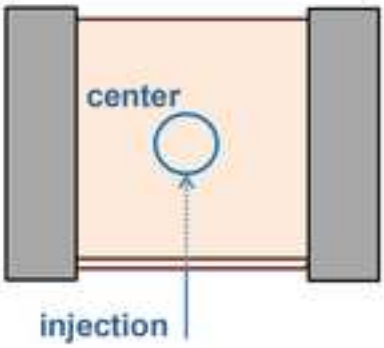


Figure2

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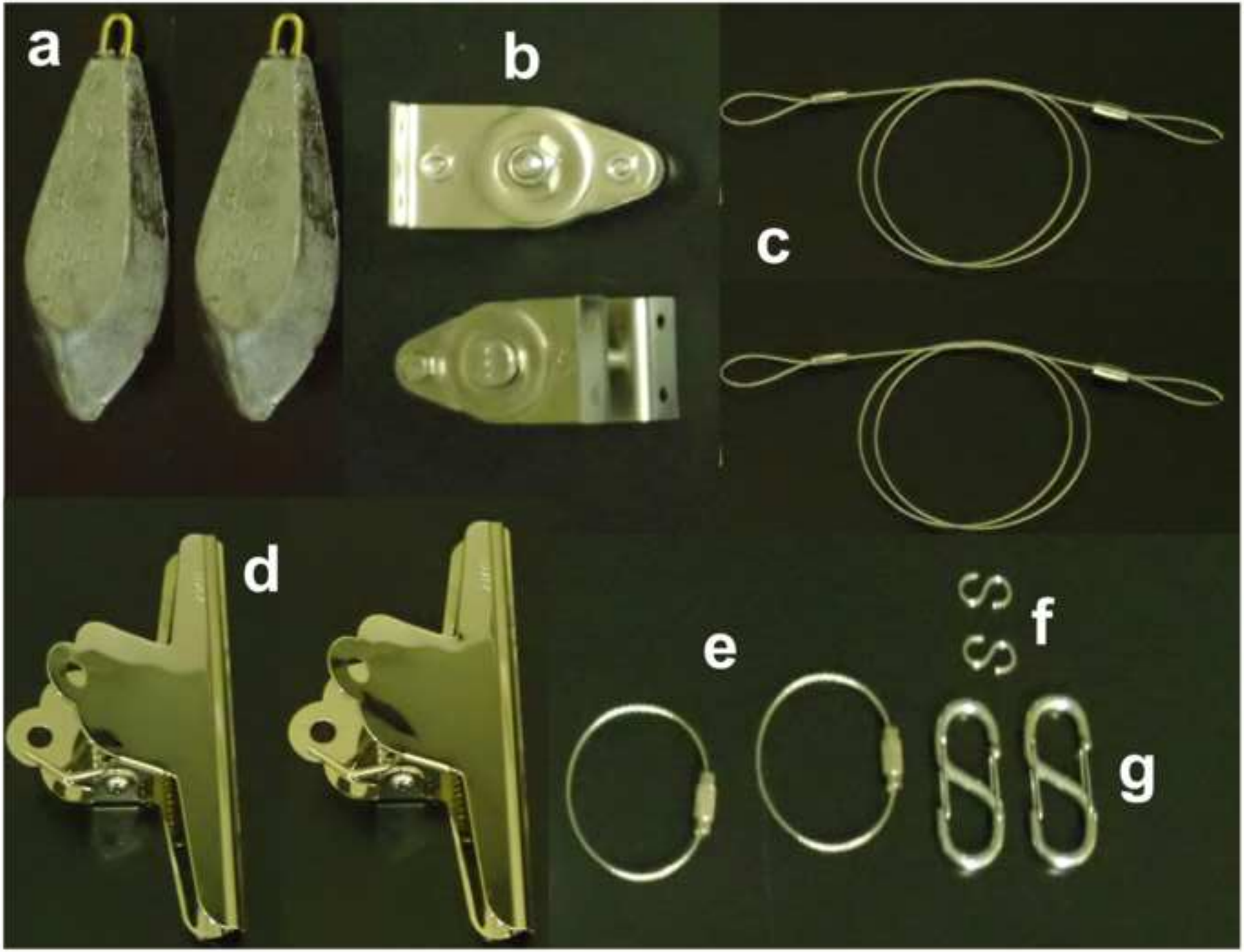

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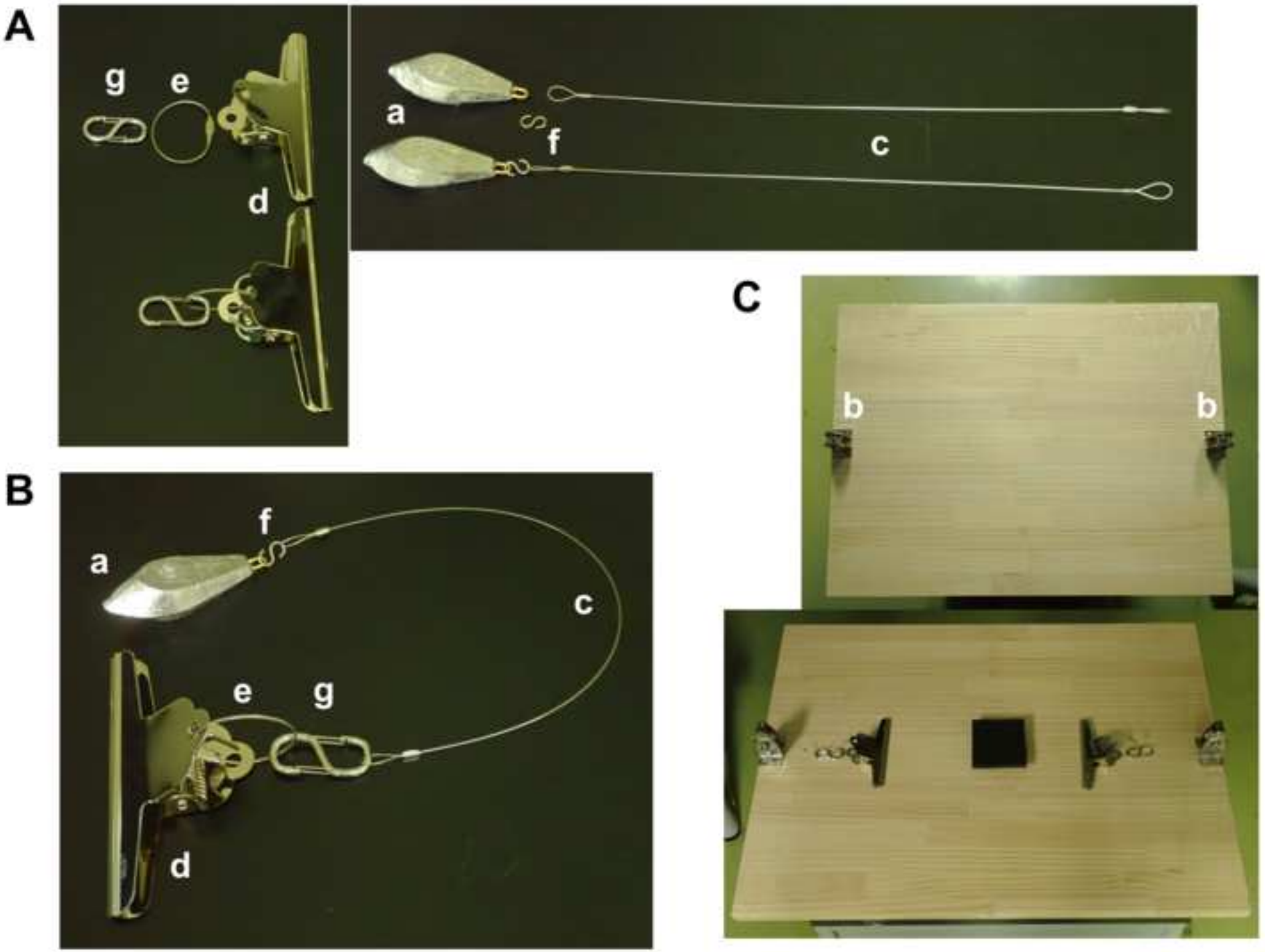

Figure4

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Figure5

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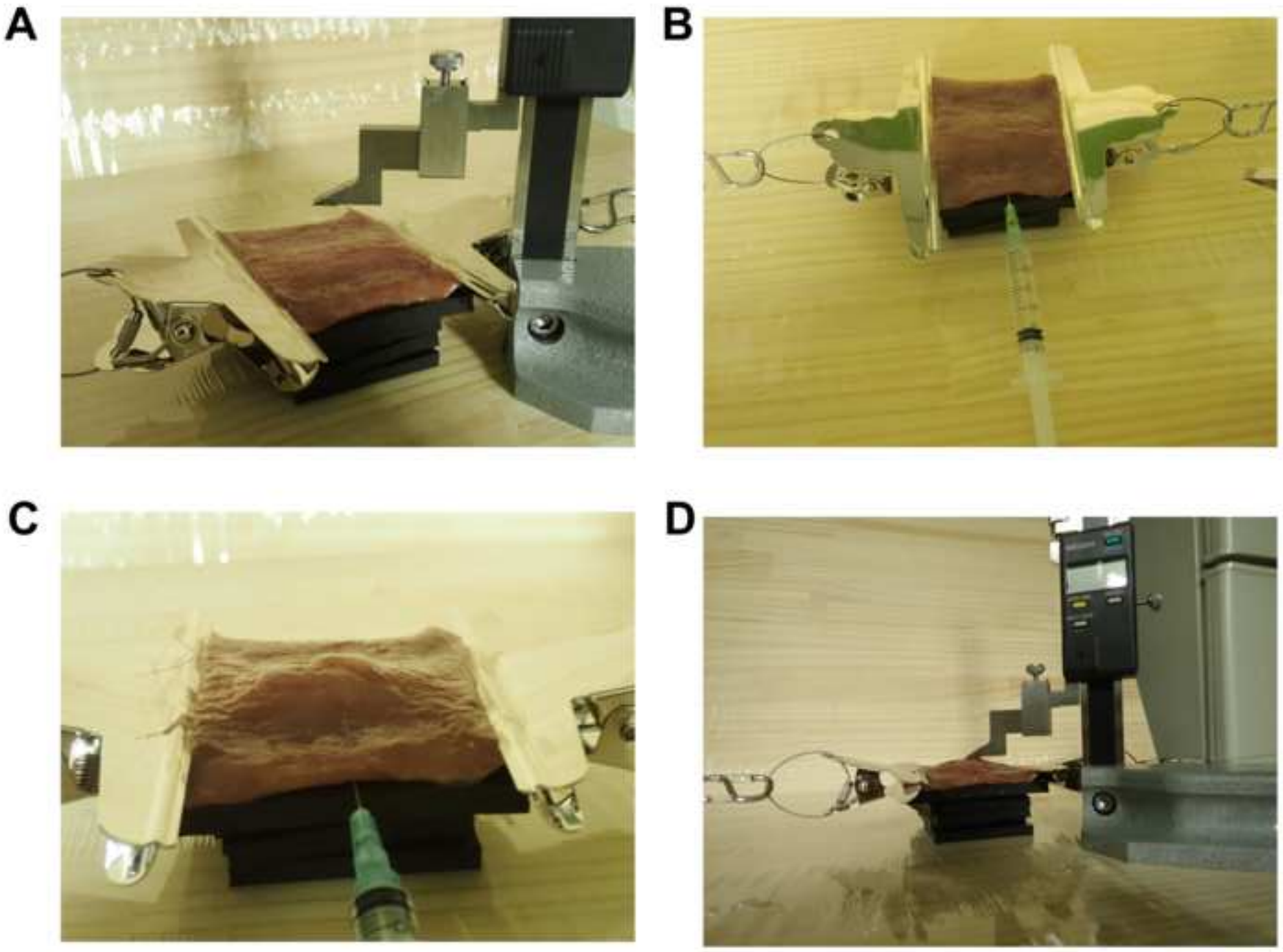

Figure6

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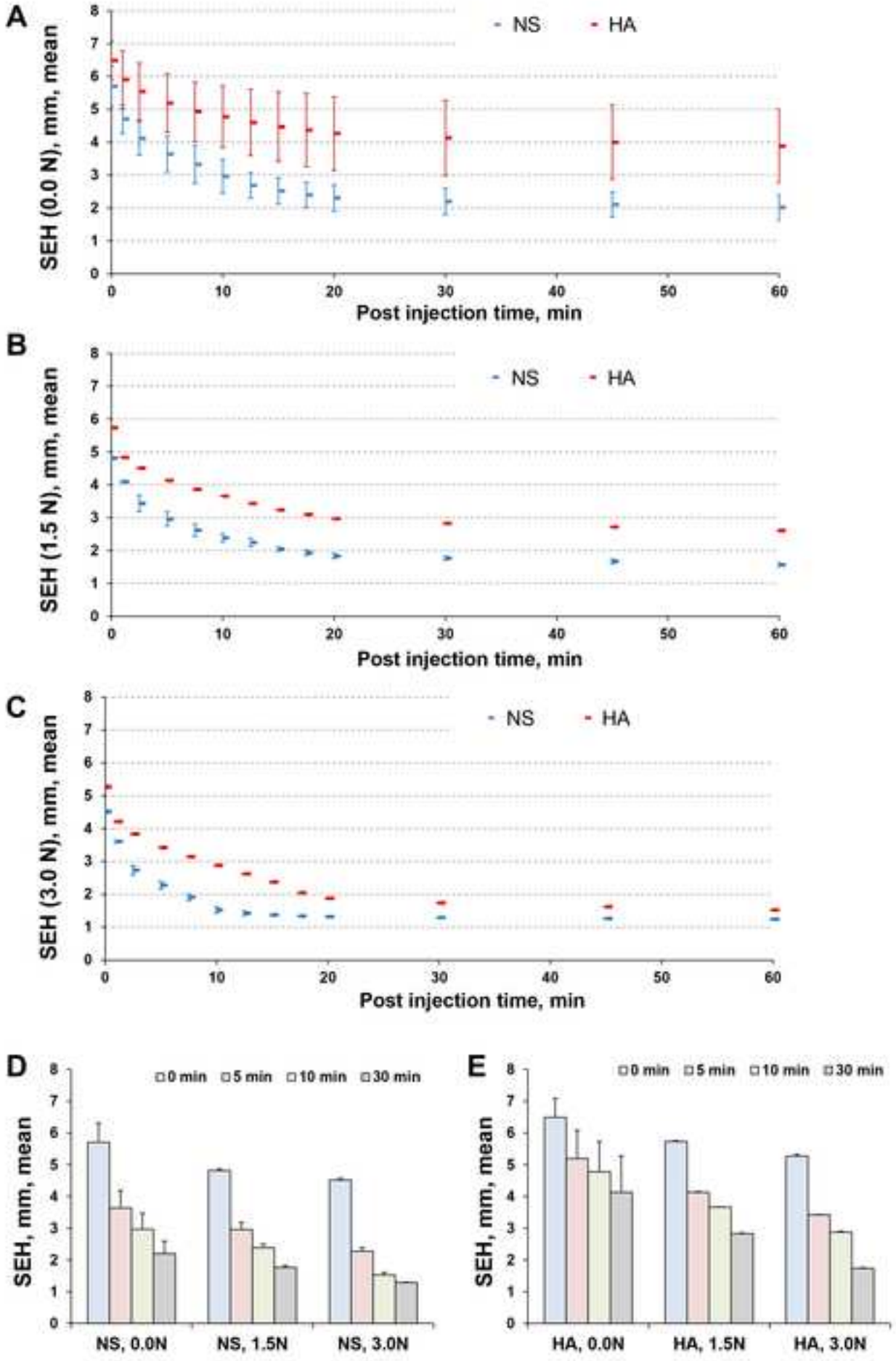

Table of Materials

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Name of Material/ Equipment	Company
weight (153.1 g)	
fixed type pulley	H.H.H. MANUFACTURING
stainless steel wire with a diameter of 0.45 mm	Nissa Chain
stainless steel clip of width 147 mm	KOKUYO
stainless steel key wire with a length of 12 cm	Nissa Chain
stainless steel S shaped hook	TRUSCO NAKAYAMA
lockable stainless steel S-shaped hook	Mizumoto Machine Mfg
rectangular wooden base (45 x 60 cm)	none
rubber plate (5 x 5 cm)	none
digital height gage	Mitutoyo
2.5-mL syringe	Terumo
23-gauge needle	Terumo
MucoUp	Boston Scientific
saline (20 mL)	Otsuka Pharmaceutical
GraphPad Prism 7 software	GraphPad Inc

Catalog Number	Comments/Description
VS25	
Cut wire Y-5	
none	
P-702	
TCS1.2	
B2054	
none	
none	
HDS-20C	
SS-02SZ	
NN-2332R	
none	0.4% sodium hyaluronate (HA)
none	normal saline (NS)
none	

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June 12, 2018

Dr. Ronald Myers, Senior Science Editor
Dr. Bing Wu, Review Editor
Journal of Visualized Experiments (JoVE)

Dear Dr. Myers and Dr. Wu:

Thank you for your kind e-mail dated Jun 06, 2018, and for the opportunity to submit a revised version of our manuscript (JoVE58029), titled "**Detailed setup methodology of a new ex vivo model for evaluation of endoscopic submucosal injection materials performance**" for your continued consideration for publication in *JoVE.*

We have carefully read the editorial comments and have revised the manuscript accordingly. Moreover, our manuscript was revised according to *JoVE* manuscript submission guidelines. We used a professional language editing service to further improve the English of our manuscript and to ensure that our meaning is clarified throughout.

We believe that we have fully addressed all the reviewers' concerns and hope that you now find the manuscript suitable for publication in *JoVE*.

Please do not hesitate to contact me with any questions or concerns.

Yours sincerely,

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