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Development of a new *ex vivo* model for evaluation of endoscopic submucosal injection materials performance

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ABSTRACT

Background and aims: Development of high-performance submucosal injection materials (SIMs) contribute to the advancement of endoscopic therapy for early-stage gastrointestinal neoplasms. This study aimed to develop a new *ex vivo* model that mimics the human gastrointestinal tract to evaluate the performance (the height and duration of the submucosal elevation) of various SIMs in detail.

Methods: A new *ex vivo* model that applies a constant tension to the tested specimen (the porcine gastric specimen) was developed. SIMs were injected into the submucosa at the center or edge of the tested specimen, and submucosal elevation heights (SEHs) were measured over time.

Results: The average value and standard deviation of SEH determined using the conventional model (the tested specimen was fixed with pins) were higher than those obtained using the new model, which showed that the new model could precisely measure the SEH of a given SIM. In addition, the performance (SEH) of SIMs decreased with increasing tension applied to the specimen, suggesting that the performance of SIMs deteriorates with the over-expansion of the gastrointestinal tract. The submucosal elevation formed at the specimen edge disappeared faster than that formed at the specimen's center.

Conclusions: The proposed new *ex vivo* model allows accurate SEH measurement under uniform conditions and detailed comparison of the performances of various types of SIMs and can contribute to the development of high-performance materials.

1. Introduction

Endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) have been widely used as treatment modalities for early-stage gastrointestinal neoplasms (stomach/esophageal/colon cancer) (Conio et al., 2006; Kantsevoy et al., 2008; Ono et al., 2001). The creation of submucosal elevation by injecting a submucosal injection material (SIM) into the submucosa is an essential step of both the EMR and ESD procedures (Conio et al., 2006; Soetikno et al., 2003). “High submucosal elevation” and “maintenance of submucosal elevation” are very important criteria for the safe and rapid EMR/ESD endoscopic treatment, which determine its success or failure in particularly difficult cases (such as large tumor size, severe fibrosis of submucosa, or sites that are difficult to treat).

Normal saline (NS) has been used as a SIM since the invention of endoscopic therapy (Iishi et al., 1997; Katsinelos et al., 2008), while the development of high-performance SIMs has been conducted until today. In recent years, sodium hyaluronate (HA), a highly viscous SIM, was introduced into the treatment (Yamamoto et al., 1999a, 1999b). It was capable of reaching and maintaining a submucosal elevation that was higher than the level achieved by injecting NS and caused almost no damage to the surrounding tissues; as a result, HA became widely used in endoscopic treatments as a superior SIM (Kishihara et al., 2012; Yamamoto et al., 2003, 2008; Yoshida et al., 2012). To identify a superior SIM, a performance comparison between the existing SIMs was conducted (Conio et al., 2002; Huai et al., 2015; Hurlstone et al., 2008; Katsinelos et al., 2008; Lee et al., 2004; Moss et al., 2010; Uraoka et al., 2005; Yandrapu et al., 2016). Furthermore, the development of high-

Abbreviation: SIM, submucosal injection material; SEH, submucosal elevation height; EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; NS, normal saline; HA, sodium hyaluronate

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performance SIMs comparable to that of HA has been performed all over the world (Fernandez-Esparrach et al., 2009; Hayashi et al., 2004; Tran et al., 2012).

Because the estimation of SEH and its duration in the actual human gastrointestinal tract under the same conditions is a difficult task, the *ex vivo* model utilizing a porcine stomach is frequently used to compare the performances of different types of SIMs (Akagi et al., 2011; Eun et al., 2007; Fernandez-Esparrach et al., 2009; Tran et al., 2012). However, since the conventional *ex vivo* model is a simple model and conditions are not perfectly uniform between each measurement, there is room for improvement. If this conventional *ex vivo* model can be further improved to reproduce an environment close to that of the human gastrointestinal tract, the height and duration of the submucosal elevation can be measured more precisely. As a result, an objective and detailed performance comparison between several types of SIMs will become possible, which will greatly contribute to the development of the next generation of such materials.

In this study, we aimed to develop a new *ex vivo* model reproducing the environment close to that of the human gastrointestinal tract, which was used to evaluate the performance (the height and duration of the submucosal elevation) of various SIMs in detail. The findings obtained in this study can provide a basis for developing the high-performance SIMs for advanced endoscopic treatment.

2. Material and methods

2.1. Development of a new ex vivo model using a porcine stomach

The thickness of the porcine gastric wall varies in different areas of the stomach. In this study, the upper third of the porcine stomach, which is similar to the human stomach, was used. Gastric specimens were cut into squares with approximate dimensions of 5 × 5 cm and immediately stored at a temperature of −30 °C. To ensure uniform analysis conditions, all frozen gastric specimens were thawed right before the analysis procedure.

The thawed specimen was stretched flat on a rubber board using three different methods. In the first method (representing the conventional model), the specimen was fixed with pins; as a result, very little tension was applied to it (Fig. 1A) (Akagi et al., 2011; Eun et al., 2007; Fernandez-Esparrach et al., 2009; Tran et al., 2012). In the second and third methods (which were used in this study), a constant tension (1.5 N or 3.0 N) was applied by fixing/stretching both ends of the specimen with a clip (Fig. 1B and C). After stretching the specimen using one of the three described methods, a SIM was injected into the submucosa at the center or edge of the specimen followed by submucosal elevation (Fig. 2). In this study, NS (Otsuka Pharmaceutical, Tokyo, Japan) and HA (MucoUp; Boston Scientific, Marlborough, MA) were used as SIMs.

The injection into the submucosal layer at the edge of the specimen reproduces the conditions of the submucosal injection during the endoscopic treatment after the mucosal incision (such as the process of submucosal resection during ESD). Hence, a new *ex vivo* model for the evaluation of submucosal elevation at the center and edge of the specimen stretched at a constant tension has been developed (Fig. 2).

Furthermore, the new *ex vivo* model consists of parts that are easily available and can be quickly set up (Fig. 3).

2.2. Evaluation of SIM performance

To evaluate the performance of various SIMs, the magnitude of SEH was measured at specified time intervals. Using a 2.5-mL syringe and 23-gauge needle, we injected 2.0 mL of each solution horizontally into the submucosa from the specimen margins and performed a submucosal elevation procedure. SEH was precisely measured with a digital height gage (HDS-20C, Mitutoyo, Kanagawa, Japan) at 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 30, 45, and 60 min after the injection. Three independent measurements were performed, and the obtained results were expressed as the mean and standard deviation.

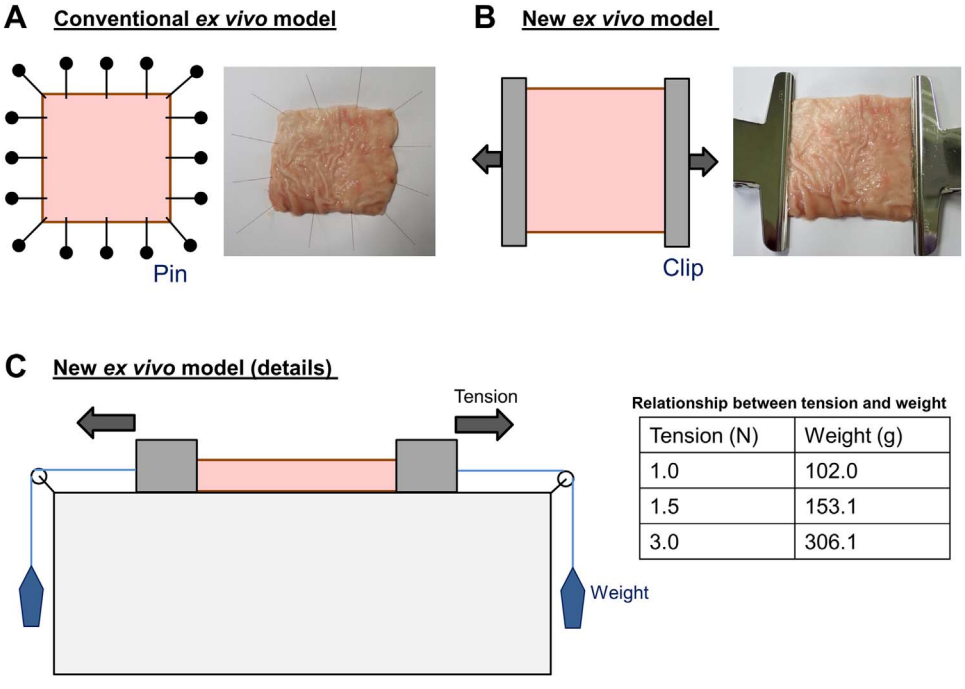
2.3. Statistical analysis

The obtained data were analyzed using the GraphPad Prism 7 software (GraphPad Inc., La Jolla, CA, USA). Continuous variables were evaluated with the Student's t-test. All the reported P values were two-sided, and the magnitudes with $P < 0.05$ were considered significant.

3. Results

3.1. Performance evaluation of SIMs using the proposed ex vivo model

In the new *ex vivo* model developed in this work, constant tension



Relationship between tension and weight

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

Fig. 1. New *ex vivo* model using the porcine stomach. A new *ex vivo* model that can apply constant tension to the specimen ends was developed. The conventional *ex vivo* model represented a simple model, in which the specimen was fixed with pins **(A)**. In the new *ex vivo* model, a constant tension can be applied to the specimen by stretching both its ends with a clip **(B)**. This model can be tensioned uniformly by using a weight **(C)**.