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ABSTRACT

Background and Aims: Endoscopic submucosal dissection (ESD) can remove varying size early stage GI tumors en bloc; however, success requires reducing a relatively high post-procedure bleeding rate. The aim of this study was to assess the safety and efficacy of a novel fully synthetic and self-assembled peptide solution which functions as an extracellular matrix scaffold material that facilitate the reconstruction of normal tissues in ESD-induced ulcers.

Methods: Consecutive patients who underwent gastric ESD were prospectively enrolled. Immediately after the resection, the solution was applied to the site with a catheter. Gastric ulcers were evaluated by endoscopy and classified as active, healing, or scarring stages at 1, 4, and 8 weeks after ESD.

Results: Forty-seven patients with 53 lesions, including 14 (29.8%) previously on antithrombotic therapy and 2 (4.3%) requiring heparin bridge therapy were analyzed and 2 patients were excluded due to 1 perforations and 1 with persistent coagulopathy. The mean size of en bloc resected specimen was 36.5 ± 11.3 mm. The rate of post-ESD bleeding was 2.0% (1/51; 95% CI, 0.03 - 10.3). Transitional rate to
healing stage of ESD-induced ulcer at week 1 was 96% (49/51). Subsequent endoscopies demonstrated scarring stage in 19% (9/48) and 98% (41/42) at 4 and 8 weeks, respectively. There were no adverse effects related with this solution.

Conclusions: The use of this novel peptide solution may potentially aid in reducing the delayed bleeding rate by promoting mucosal regeneration and speed of ulcer healing after large endoscopic resections in the stomach. Further studies, particularly randomized controlled type are needed to fully evaluate its efficacy.

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UMIN Clinical Trials Registry: 000011548

Introduction

Endoscopic submucosal dissection (ESD) can remove varying size
early stage GI tumors en bloc, which can be curative or at least reduce local recurrence and facilitate more accurate histopathological assessment.\textsuperscript{1-3} However, delayed bleeding can occur days after the procedure and success requires reducing a relatively high postprocedure bleeding rate particularly in the stomach; (4.6 to 15.6\%).\textsuperscript{4-7} Although gastric ESD-induced ulcers are treated with proton pump inhibitors (PPIs) for at least 8 weeks in most hospitals, it does not eliminate the risk of bleeding.\textsuperscript{8} Prevention of post-ESD bleeding is important as it can potentially result in significant morbidity from acute blood loss and need for additional endoscopic intervention and extended hospitalization.

Recently, tissue engineering and regenerative substances, such as extracellular matrix scaffold material (ECM-SM) have been developed to target the reconstruction of structurally and functionally normal tissues.\textsuperscript{9} In addition, a fully synthetic and self-assembled peptide solution, which functions like ECM-SM to replace collagen has been developed.\textsuperscript{10-11}

We conducted this clinical trial with the aim to assess the safety and efficacy of this novel peptide solution for management of
ESD-induced gastric ulcers.

Methods

The study protocol was approved by the institutional review board of the Keio University hospital. Consecutive patients, who underwent ESD for intraepithelial gastric tumors\textsuperscript{12} by 8 endoscopists of various level of experience, were enrolled.

Patients receiving antithrombotic therapy were included in this study however, this was discontinued before the procedure in patients low risk for thromboembolism according to current guidelines by the Japan Gastroenterological Endoscopy Society.\textsuperscript{13} Heparin bridge therapy (HBT) was administered to patients who were considered high risk for thromboembolism until 6 hours before ESD, and restarted on the first postprocedure day. Patients with platelet count <50,000/mm\textsuperscript{3} were excluded in this trial. Additionally, cases with coagulopathy (INR >2) despite appropriate management and those complicated with perforations were excluded from the analysis.

Informed signed consent for the procedure and use of the peptide solution was obtained from all patients.
**Fully-synthetic and self-assembled peptide solution**

A novel fully-synthetic material consisting of 16-amino acid peptides solution (PuraMatrix, 3-D Matrix, Ltd, Tokyo, Japan) self-assembles at physiological pH and forms a hydrogel comprising a network of nanofibers. This rapidly seals open blood vessels when exposed to blood or tissue fluids. In addition, the important features of PuraMatrix includes its nano-structure that is equivalent to natural ECM-SM (nano-fiber,) which results in adequate adherence of the cells and tissue.¹⁴

**Study protocol**

For every 1 cm of resected tumor, 1 mm³ of PuraMatrix was immediately applied to the resection site using a catheter (TOP Endoscopic Spraying Tube, Top, Co, Ltd., Tokyo, Japan). All patients received a single dose proton pump inhibitor (PPI) for 8 weeks beginning on the morning of the procedure.

Post-ESD bleeding was defined as that requiring endoscopic or surgical intervention, or drop in hemoglobin by 2 g/dL and hemodynamic
instability. Images of ESD-induced ulcers were collected prospectively and digitally stored and post-ESD gastric ulcer stages were evaluated by endoscopy and classified as active, healing, or scarring at week 1, 4, and 8, respectively. The healing stage of gastric ESD-induced ulcer was defined as an ulcer without mucous coating and margin elevation according to the Sakita and Fukutomi classification. This was classified at follow-up endoscopy after careful inspection and thorough review by 2 experienced endoscopists who did not participate in the ESD procedure. Although this classification is generally used to assess the healing process of the peptic gastric ulcer, we used this classification for ESD-induced ulcer in this study because it is the only most objective guide published. When there were any differences in opinion between their respective stages, the 2 endoscopists discussed their findings and agreed upon a mutually agreeable and best staging. Although the Sakita and Fukutomi classification was directly related to peptic ulcer disease and the pathophysiology of its cause is certainly different from that of an iatrogenic induced ulcer/mucosal defect after ESD, tissue regeneration and healing is by the same process from a
histologic perspective. Therefore, given the classification is the only objective staging guide available, it was used in this study to reduce interobserver interpretation variability.

The primary endpoint was the rate of post-ESD bleeding. The secondary endpoints include the transitional rate to healing and scarring stages of gastric ESD-induced ulcers.

**Statistical Analysis**

The continuous values were expressed as mean ±SD. JMP, version 8, software (SAS Institute, Cary, NC) was used to analyze data and the significance level was set at 5% for each analysis so a p value <0.05 was considered statistically significant.

**Results**

**Baseline characteristics**

A total of 47 patients were recruited and 45 patients with 51 lesions were enrolled for outcome analysis. Two patients were excluded due to perforation and coagulopathy. Of the 45 patients, 14 (29.8%) were previously on antithrombotic therapy including 2 (4.3%) requiring
HBT. En bloc resection was achieved in all cases. The mean size of resected specimen was 36.5 ± 11.3 mm.

**Post-ESD bleeding**

Post-ESD bleeding was observed in 1 cases (1/51, 2.0% [95% CI, 0.03 - 10.3]). The only post-ESD bleeding case was successfully managed by endoscopic intervention alone.

**Transitional rates to stages of gastric ESD-induced ulcers**

A total of 51 gastric ESD-induced ulcers had scheduled follow-up endoscopy at 1, 4, and 8 weeks after ESD. Complete follow-up was not possible in 8 cases, 4 of which underwent surgery for non-curative resection and 2 requiring pyloric ring dilation for stricture management during the follow-up period. The other 2 patients declined subsequent endoscopy.

Transitional rate to healing stage at 1 week was 96% (49/51). Further follow-up gastroscopies demonstrated scarring stage in 19% (9/48) and 98% (41/42) at weeks 4 and 8, respectively. *(Table 2) (Figure 1)*
There were no adverse effects related with PuraMatrix use in this study.

Discussion

In this feasibility study, the application of a novel fully synthetic and self-assembled peptide solution, PuraMatrix, demonstrated its ability to reduce delayed bleeding rate and promote mucosal regeneration and speed of ulcer healing after large endoscopic resections in the stomach. The rate of bleeding was 2.0% (1/51). These results reveal lower post-ESD bleeding rates than previous reports.\textsuperscript{4–7}

The synthetic peptide solution was tested as a hemostatic material in pilot studies with small number of cases demonstrating hemostasis of the active oozing/bleeding during gastric ESD and to vascular anastomotic graft sites.\textsuperscript{16,17} Both studies demonstrated more effective and reliable hemostasis than other commonly used general hemostatic agents. In addition, Kondo et al reported the safety and effectiveness of PuraMatrix as a bio-compatible sealing material for the management of post-operative peritoneal effusion after pelvic surgery.\textsuperscript{18} There are no reports about this solution for promoting wound healing by
sequential observation in any of the medical fields.

Recently, a shielding method using polyglycolic acid (PGA) sheets and fibrin glue for gastric ESD-induced ulcer to prevent post-ESD bleeding in high-risk patients had been reported by Tsuji et al.\textsuperscript{19} They reported decreased post-ESD bleeding rate (6.7\%) in high-risk patients who were taking antithrombotic drugs (antithrombotic drugs were discontinued pre-procedure) or were expected to undergo large mucosal resection (≥40 mm). When compared with our gastric ESD cases, mean resected specimen size of 36.5 mm and 21\% patients with current use of antithrombotic agents, their post-ESD bleeding rate was not superior. The shielding method using PGA sheets and fibrin glue has some disadvantages, which include risks of unknown infection and longer procedure time. According to their report, the mean procedure time for applications of the sheets and glue was 20.4 ± 9.5 minutes. Because PuraMatrix is a fully synthetic solution, there are no concerns for the risk of infection. Also, the application of PuraMatrix using a catheter is completed within a minute.

Gastric ESD-induced ulcers were specifically evaluated after ESDs. Because the risk of delayed bleeding is highest in gastric ESDs than
esophageal or colorectal ESDs despite the routine use of PPIs, the evaluation of gastric ESD-induced ulcers is clinically important to reduce morbidity. When comparing previous reports, the rate of the healing stage at 1 week and the rate of scarring at 4 weeks were higher. (Table 2) In addition, the rate of scarring even at 8 weeks was relatively higher in our study than previous reports. PuraMatrix seems to promote ulcer healings according to these comparisons because objective assessment was done according to the Sakita and Fukutomi classification.

The advantages of PuraMatrix for endoscopic resection-induced ulcers as follows: (1) function as synthetic “ECM” to replace collagen, tissue engineering and regenerative substances, (2) no risk of infection because it being a fully synthetic solution, (3) a transparent aqueous solution that rapidly forms into hydrogel in the body at physiologic pH, and (4) an aqueous solution with easy application through a catheter from a prefilled syringe. This study has some limitations that include a single-center study, the relatively small number of patients, and the non-randomized protocol of the investigation. Also, in this study, we used a standard catheter
but have been developing a special delivery catheter and are expecting to achieve more effectiveness of PuraMatrix by better application through the use of this novel catheter.

In conclusion, the use of this novel fully synthetic and self-assembled peptide solution may potentially help reduce rate of post-ESD bleeding and promote ulcer healing for ESD-induced gastric ulcers. Further studies, particularly randomized controlled type, are needed to fully evaluate its efficacy. Therefore, a multicenter, randomized controlled trial should be seriously considered to fully evaluate the efficacy of this novel peptide solution.

REFERENCES


vessels may prevent delayed bleeding after endoscopic submucosal dissection—an analysis of risk factors. Endoscopy 2008;40:179-83


**Table 1: Baseline Characteristics**

<table>
<thead>
<tr>
<th>Patients</th>
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<tbody>
<tr>
<td>Age, mean (S.D.)</td>
<td>71.9 (8.8)</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>73.3</td>
</tr>
<tr>
<td>Current use of antithrombotic agents, n(%)</td>
<td>14 (29.8%)</td>
</tr>
<tr>
<td>Administration of heparin bridge therapy, n(%)</td>
<td>2 (4.3%)</td>
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</table>

<table>
<thead>
<tr>
<th>Lesion</th>
<th></th>
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<tbody>
<tr>
<td>Stomach: U/M/L</td>
<td>16/17/18</td>
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</table>

<table>
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<tr>
<th>Outcome of ESD</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>En bloc resection (%)</td>
<td>51 (100)</td>
</tr>
</tbody>
</table>

S.D., standard deviation

Upper third of the stomach; M, middle third of the stomach; L, lower third of the stomach.
Table 2: Comparison of transitional rates to stages of gastric ESD-induced ulcers with proton pump inhibitor treatment

<table>
<thead>
<tr>
<th>Mean Resected Size</th>
<th>1 Week</th>
<th>4 weeks</th>
<th>8 Weeks</th>
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<td></td>
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</table>
| Park et al.⁹)      | 39.1 mm| N/A     | N/A     | Active 0%  
|                    |        |         |         | Healing 31%  
|                    |        |         |         | **Scarring 69%** |
| Uedo et al.¹⁰)     | 41.0 mm| N/A     | N/A     | Active 0%  
|                    |        |         |         | Healing 17%  
|                    |        |         |         | **Scarring 83%** |
| Ye et al.¹¹)       | N/A    | N/A     | Active 4% | N/A  
|                    |        |         |         | Healing 92%  
|                    |        |         |         | **Scarring 4%** |
| Kawano et al.¹²)   | N/A    | N/A     | Active 3% | Active 0%  
|                    |        |         |         | Healing 85%  
|                    |        |         |         | **Scarring 11%**  
|                    |        |         |         | **Scarring 89%** |
| Kakushima et al.¹³) | 34.7 mm| Active 100% | Active 0% | Active 0%  
|                    |        |         |         | Healing 99%  
|                    |        |         |         | **Scarring 1%**  
|                    |        |         |         | **Scarring 100%** |
| Our study          | 36.5 mm| Active 4% | Active 0% | Active 0%  
|                    |        |         |         | Healing 96%  
|                    |        |         |         | **Scarring 19%**  
|                    |        |         |         | **Scarring 98%** |

N/A, not applicable
FIGURE LEGENDS

Figure 1 – Transition of gastric ESD-induced ulcers in the gastric angle after ESD.

A. An early stage cancer on the posterior of gastric angle.

B. Endoscopic image after acetic acid and indigo-carmine dye spay.

C. ESD-induced ulcer immediately after ESD

D. An application of PuraMatrix

E. One week after ESD, ulcer was at healing stages according to Sakita and Fukutomi classification

F. At 4 weeks, the scarring stage was observed.

G. At 8 weeks, the scarring stage was observed.
Author contributions:

Toshio Uraoka: study concept and design, data collection and analysis and writing of manuscript.

Yasutoshi Ochiai, Ai Fujimoto and Osamu Goto: intellectual contribution and data collection.

Yoshiro Kawahara, Naoya Kobayashi, Takanori Kanai, Sachiko Matsuda Yuko Kitagawa: intellectual contribution.

Naohisa Yahagi: intellectual contribution, data collection and final approval of the article.
**Abbreviations:** ESD, endoscopic submucosal dissection; GI, gastrointestinal; PPI, proton pump inhibitor; ECM-SM, extracellular matrix scaffold material; HBT, heparin bridge therapy; PGA, polyglycolic acid