Safe and Efficient Colonic Endoscopic Submucosal Dissection Using an Injectable Hydrogel

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ABSTRACT: Endoscopic submucosal dissection (ESD) has not yet been widely adopted in the treatment of early colonic cancers due to the greater technical difficulty involved, longer procedure time, and the increased risk of perforation. Adequate mucosal elevation by submucosal injection is crucial for en bloc resection and prevention of perforation during colonic ESD. This study is aimed to evaluate the efficacy of an injectable thermoreversible hydrogel as the colonic submucosal agent for the first time. Triblock copolymer poly(lactic acid-co-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-co-glycolic acid) (PLGA–PEG–PLGA) was synthesized, and its concentrated aqueous solution was injected into the colonic submucosa of living minipig and spontaneously transformed into an in situ hydrogel with adequate mucosal elevation at body temperature. Such a mucosal lifting lasted for a longer time than that created by the control group, glycerol fructose. Colonic ESD was then performed with the administration of hydrogels at various polymer concentrations or glycerol fructose. All colonic lesions were successfully resected en bloc after one single injection of the hydrogel, and repeated injections were not needed. No evidence of major hemorrhage, perforation and tissue damage were observed. Considering the injection pressure, duration of mucosal elevation and efficacy of “autodissection”, the hydrogel containing 15 wt % polymer was the optimized system for colonic ESD. Consequently, the thermoreversible hydrogel is an ideal submucosal fluid that provides a durable mucosal lifting and makes colonic ESD accessible to a large extent. In particular, the efficacy of “autodissection” after one single injection of the hydrogel simplifies significantly the procedures while minimizing the complications.

KEYWORDS: endoscopic submucosal dissection (ESD), submucosal injection agent, injectable hydrogel, colonic tumor, autodissection

INTRODUCTION

Colorectal cancer becomes one of major causes of morbidity and mortality in both Western countries and Eastern counties. For many years, endoscopic mucosal resection (EMR) and surgery had been the only available modalities in the treatment of colorectal tumors, at least in Western countries. However, for large tumors, an en bloc resection by EMR is difficult and the chance of piecemeal resection is high. Separate resection of large tumors, an en bloc resection by EMR is difficult. A continuous use of electrocautery to resect submucosa in colorectal ESD is substantially higher than that in gastric ESD, and the perforation in the colon can cause fatal peritonitis. Injection of various fluids into the submucosa to lift the lesion away from the muscularis propria layer of the gut is considered as one of the most effective approaches to avoid perforation or excessive hemorrhage yet achieve en bloc resection during ESD. Normal saline (NS) is commonly used for this purpose. However, the duration of mucosal elevation after NS injection is very short because of the rapid absorption of NS by the surrounding tissue, thereby requiring repeated injections for larger lesions, which results in the consuming because of the thin wall and winding nature, especially of the colon, and endoscopic stabilization in the colon is more difficult than in gastric ESD. It has been reported that the average procedure time of ESD for colorectal lesions ranged between 70 and 110 min. Meanwhile, because of its higher difficulties, the rate of perforation that comes from the continuous use of electrocautery to resect submucosa in colorectal ESD is substantially higher than that in gastric ESD, and the perforation in the colon can cause fatal peritonitis.

Injection of various fluids into the submucosa to lift the lesion away from the muscularis propria layer of the gut is considered as one of the most effective approaches to avoid perforation or excessive hemorrhage yet achieve en bloc resection during ESD. Normal saline (NS) is commonly used for this purpose. However, the duration of mucosal elevation after NS injection is very short because of the rapid absorption of NS by the surrounding tissue, thereby requiring repeated injections for larger lesions, which results in the consuming because of the thin wall and winding nature, especially of the colon, and endoscopic stabilization in the colon is more difficult than in gastric ESD. It has been reported that the average procedure time of ESD for colorectal lesions ranged between 70 and 110 min. Meanwhile, because of its higher difficulties, the rate of perforation that comes from the continuous use of electrocautery to resect submucosa in colorectal ESD is substantially higher than that in gastric ESD, and the perforation in the colon can cause fatal peritonitis.
increased time of procedure. To overcome this shortcoming, some agents holding high viscosity, such as hyaluronic acid, glycerol fructose, hydroxypropyl methylcellulose, and sodium alginate, have also been investigated for mucosal elevation. Nevertheless, these agents showed limited efficacy and has not been widely adopted in clinic so far. Therefore, to achieve the easier, safer, and faster colonic ESD procedure, development of more effective submucosal injection agents is still desired.

Over the past decade, injectable polymeric hydrogels have been drawing attention as implanted biomaterials because of easy administration, minimally invasiveness, site specific introduction and facile formation of three-dimensional (3D) morphology under mild conditions. In particular, thermoreversible hydrogels that are in the sol state at ambient temperature but the gel state at body temperature could be injected into body in the sol state and then spontaneously form semisolid gels at the injection site. As a typical example, thermoreversible poly(lactic acid-co-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-co-glycolic acid) (PLGA-PEG-PLGA) hydrogel is composed of a linear triblock copolymer of hydrophilic PEG segment and biodegradable PLGA block. As well-known, both PEG and PLGA have been approved by Food and Drug Administration and relevant bureau, and clinically utilized in many countries. To date, thermoreversible PLGA-PEG-PLGA hydrogels have been extensively investigated as the sustained drug delivery depots, antiadhesion barriers and injectable tissue engineering scaffolds. However, to the best of our knowledge, none of the hydrogels has ever been utilized as a submucosal injection agent in colonic ESD, which is of more difficulty of technique and higher risk of perforation. It is thus much desired to know whether the assistance of hydrogel can facilitate colonic ESD procedures, reduce complications, and extend colonic ESD to a larger extent.

In the present study, we employed the thermoreversible PLGA-PEG-PLGA hydrogel, a unique injectable physical hydrogel, as a demonstration of medical hydrogels potentially used in colonic ESD, as schematically presented in Figure 1. The feasibility, safety, and efficacy of the thermoreversible PLGA-PEG-PLGA hydrogel as a submucosal injection substance for colonic ESD were evaluated in living porcine models. The hydrogels with various polymer concentrations were examined and the optimized hydrogel system was achieved.

**MATERIALS AND METHODS**

**Synthesis of PLGA-PEG-PLGA Triblock Copolymers.** Triblock copolymer PLGA-PEG-PLGA was synthesized by the ring-opening polymerization of monomers (D,L-lactide (LA) and glycolide (GA)) in the presence of PEG using stannous octoate as the catalyst. The detailed synthesis procedure was described in our previous publication. In brief, 30 g of PEG of molecular weight (MW) 1500 was first stirred and dried under vacuum at 130 °C in a four-neck flask for 3 h to remove the residual moisture in the polymers. Then, LA and GA with the indicated amounts are added and heated under vacuum at 100 °C for 0.5 h. After all the monomers were melted, the catalyst, Sn(Oct)2 (0.2 wt % of monomers), was transferred into the reaction mixture. Subsequently, the reaction system was maintained at 150 °C with continuous stirring for 12 h under an argon atmosphere. After completion of the reaction, a vacuum was used for 3 h to remove unreacted monomers in the reaction mixture. Next, crude products were washed with 80 °C water for three times and the residual water in

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**Figure 1.** (a) Molecular structure of PLGA-PEG-PLGA triblock copolymer. (b) Photographs of the polymer solution (15 wt % in NS) containing indigo carmine exhibiting a sol at room temperature and a gel after heated to body temperature. The indigo carmine was used as the color agent for visualization of the procedure. (c) Schematic diagram showing the creation of submucosal fluid cushion (SFC) following the submucosal injection of thermoreversible hydrogel.
the polymers were eliminated via freeze-drying. The final polymers were collected and stored at −20 °C before use. The yield of products was about 85%.

**Physicochemical Characterization.** The chemical composition and average MW of PLGA–PEG-PLGA polymers were determined by 1H NMR measurements (Bruker, AVANCE III HD 400 MHz spectrometer) using CDCl3 as solvent. The MW distribution of copolymers was also confirmed by gel permeation chromatography (GPC, Agilent 1260) with tetrahydrofuran as solvent at a flow-rate of 1.0 mL/min at 35 °C. Monodisperse polystyrene was used to obtain the standard curve. The phase diagram of PLGA-PEG-PLGA polymers in NS were confirmed by a test tube inverting approach.4,49 A dynamic stress-controlled rheometer (Kinexus Pro, Malvern) equipped with a conical plate (diameter: 60 mm, conical angle: 1°, gap: 0.03 mm) was also used to investigate the rheological properties of the thermoreversible hydrogels with different polymer concentrations. 1.5 mL of polymeric solution in NS was added to the conical plate at low temperature and the edge of conical plate was overlaid with a layer of low viscosity silicone oil to minimize the evaporation of solvent. The complexed viscosity was collected as a function of temperature from 15 to 45 °C with a heating rate of 0.5 °C/min and a frequency of 10 rad/s. During the measurement process, strain was kept within the linear viscoelastic range.

**Creating Submucosal Cushion in Living Porcine Colon.** The experimental protocols were approved by the Animal Care and Use Committee of Zhongshan Hospital, Fudan University. The PLGA-PEG-PLGA copolymers were first dissolved in NS to obtain the polymeric aqueous solutions with different polymer concentrations (5, 10, 15, and 20 wt %). Then, the polymer solutions with given concentrations (further dilution using NS was not carried out) were directly injected into the submucosal layers of the colon using an injection needle (NM-4L-1, Olympus Medical Systems Co, Tokyo, Japan) in a living porcine model. A total of 5 mL of solution was used for each injection. The same volume of glycerol fructose was injected as the control group. All of the injection agents contained a small amount of indigo carmine (0.1 mℓ per 10 mL of solution) as a color agent. The maximum injection pressure for each injection was measured using a pressure gauge (Encore 26 Inflation Device, Boston Scientific, USA). Change of the mucosal lifting induced with various injection agents was assessed at 0, 5, 15, and 30 min postinjection. All data were recorded, and the injection site was inspected to evaluate the properties of the injection agents and their corresponding suitability of mucosal elevation. The experiment was repeated twice.

**Colonic ESD.** A standard colonscope (CF-H240, Olympus) was used in all procedures. A transparent cap (D-201–13404, Olympus) was attached to the tip of the endoscope to provide a constant endoscopic view. Other equipment included injection needle (NM-4L-1, Olympus), insulation-tripped (IT) knife (KD-620LR, Olympus), Coagrasper (FD-410LR, Olympus), hot biopsy forceps (FD-410LR, Olympus), clips (HX-610-90, HX-600-13S, Olympus), high-frequency generator (VIO 200D, ERBE), and argon plasma coagulation unit (APC300, ERBE). Two liters of a PEG-electrolyte solution was used to bowel preparation and then colonic ESD was performed under general anesthesia. In detail, after administration of injection agents into the submucosa, a circumferential precutting of the mucosal and submucosal layer around the lesion was performed. Next, the submucosal tissue underneath the lesion was carefully dissected using IT knife under direct vision. The injection agent was injected repeatedly during the dissection when necessary. Metallic clips were used to close the deeply dissected areas or small perforation. In each group, the resected specimen was about 2.0 cm × 2.0 cm.

During the procedure, the mucosa precutting and submucosal dissection were carried out using ENDO CUT Q (ERBE Surgical Systems, Inc., Marietta, GA) at 50 W, effect 3. Minor bleeding was often treated by force coagulation (60 W) using the knife tip. Pulsating bleeding from larger vessels was generally grasped and coagulated with a hemostatic forceps using a forced coagulation mode at 50 W. When large vessels were visible during the operation, they were precoagulated using hemostatic forceps in the forced coagulation mode at 50 W.

A nonsurvival animal study was conducted to examine the feasibility of “autodissection” property after injection of the thermoreversible hydrogel during the colonic ESD. A submucosal injection of each injection agent (10 wt % hydrogel, 15 wt % hydrogel, 20 wt % hydrogel, or glycerol fructose) was performed and a circumferential mucosal incision was accomplished. Then, the endoscope was tried to advance into the submucosal space to provide slight blunt force. A good “autodissection” property was noted when the submucosal tissues were autodissected just by slight blunt force from the endoscope, and there was almost no need to perform any colonic submucosal dissection except for coagulation of some big vessels. The “autodissection” property was evaluated and compared under direct view. ESD procedure was repeated three times for each injection agent in 3 pigs. Immediately following completion of all ESD procedures, the animals were euthanized and necropsy was performed. The ESD wounds were carefully inspected to determine persistence of submucosal injection agent and to evaluate tissue damage and perforation.

After the nonsurvival study, we proceeded to the survival experiment in porcine models to evaluate the safety and efficiency of 15 wt % hydrogel compared with glycerol fructose for submucosal injection during colonic ESD. Using the aforementioned ESD technique, a total of 3 colonic ESDs were performed for each group in 3 pigs. After ESD was performed, the pigs recovered from general anesthesia. They were observed clinically for evidence of delayed bleeding and perforation. After 1 week, they underwent endoscopic re-examination to inspect each operation site to evaluate for tissue damage and persistence of residual submucosal injection agent. The animals were then sacrificed, and necropsy was performed to investigate delayed tissue damage and perforation.

**Pathological Evaluation.** Tissues and resected specimens were fixed with 4% neutral buffered formalin and embedded in paraffin. Histological sections were stained with hematoxylin-eosin (HE), and observed on a light microscope (Axiovert 200, Zeiss).

**Outcome Measurements.** The main outcome measures were (1) the feasibility, safety, and durability of SFCs created with the hydrogels; (2) the “autodissection” properties of the hydrogels during colonic ESD; and (3) the comparison of en bloc resection rate, procedure-related parameters, and complications between 15 wt % hydrogel group and glycerol fructose group.

**Statistical Analysis.** Statistical analysis was performed with SPSS 17.0 software (SPSS, Chicago, IL). Measurement values were expressed as the mean and standard deviation values. Statistical significance was evaluated using Student’s t test or Fisher’s exact test as appropriate. All reported P values were two-tailed, and P values of less than 0.05 were considered to indicate statistical significance.

## RESULTS

**Synthesis and Characterization of PLGA-PEG-PLGA Triblock Copolymers.** Triblock copolymer PLGA-PEG-PLGA was prepared via a typical ring-opening copolymerization of LA and GA using the hydroxyl-terminated PEG as the initiator in the presence of stannous octoate as the catalyst. The 1H NMR spectrum of the PLGA-PEG-PLGA triblock copolymer is presented in Figure 2 and all the characteristic peaks were well assigned. On basis of the previous publications,47,50 both the number-average MW of PLGA-PEG-PLGA triblock copolymer and LA/GA molar ratio were determined via the integration of the peaks at 1.55, 3.60, and 4.80 ppm, which were assigned to methyl protons of the LA units, methylene protons of the GA units and PEG, respectively. GPC analysis further demonstrated that the polymer exhibited a unimodal distribution with dispersity of 1.22, indicating that the desired sample was obtained. Table 1
summarizes the molecular parameters of PLGA-PEG-PLGA triblock copolymer.

Table 1. Characterization of PLGA-PEG-PLGA Triblock Copolymer Used in This Study

<table>
<thead>
<tr>
<th>sample</th>
<th>(M_n^{a})</th>
<th>LA/GA(^{a})</th>
<th>(M_n^{b})</th>
<th>((M_w^{b}/M_n^{b}))</th>
</tr>
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<tbody>
<tr>
<td>PLGA-PEG-PLGA</td>
<td>1650–1500–1650</td>
<td>11.7</td>
<td>9100</td>
<td>1.22</td>
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\(^a\)The number-average MW, \(M_n\), of the central block PEG was provided by Aldrich. The \(M_n\) of each PLGA block and molar ratio of LA/GA were calculated by \(^1\)H NMR. \(^b\)Determined via GPC analysis.

Properties of Thermoreversible PLGA-PEG-PLGA Hydrogels. Figure 3a shows the phase diagram of PLGA-PEG-PLGA polymers in NS. When the polymer concentration was higher than the critical gel concentration (CGC), the aqueous polymer solution exhibited a sol–gel–sol (suspension) transition with changing temperature. Otherwise, the polymer/water system was just in a sol state. Figure 3b presents the change in complex viscosity of polymer aqueous solutions with various concentrations as a function of temperature. At low or room temperatures, the complex viscosity was low for all the polymer/water systems, indicating their good injectability. The abrupt elevation of complex viscosity represented the formation of semisolid hydrogels as the temperature increased. Because its concentration is lower than that of CGC, the complex viscosity of the 5 wt % PLGA-PEG-PLGA system was too low to form a nonflowing hydrogel at any temperature. These findings are well-consistent with the results illustrated in the phase diagram.

Feasibility and Durability for Submucosal Injection. In the living porcine models, a series of SFCs were created in the colons after submucosal injection of different samples, as presented in Figure 4. The height and duration of mucosa lifting were dependent on the concentration of polymers. Adequate and long-lasting mucosa lifting was achieved after injection of the 15 and 20 wt % PLGA–PEG-PLGA hydrogels.

Figure 3. Properties of thermoreversible hydrogels. (a) Phase diagram of the PLGA-PEG-PLGA polymers in NS. (b) Change in complex viscosity of the PLGA-PEG-PLGA polymers with various concentrations in NS as a function of temperature. Heating rate: 0.5 °C/min; oscillation frequency: 10 rad/s.
In contrast, the height of elevation induced with the 5 wt % PLGA–PEG-PLGA aqueous solution and the control group, glycerol fructose, had thoroughly collapsed at 5 min post-injection. On the other hand, the maximum injection pressure also increased correspondingly as the polymer concentration increased, as shown in Figure 5.

**Figure 5.** Maximum injection pressures of the thermoreversible PLGA–PEG-PLGA hydrogels with various polymer concentrations (10, 15, and 20 wt %) and the control group, glycerol fructose. The pressure value was determined using an Encore 26 inflation device (Boston Scientific, USA), as shown in inset.

Histologic examination further demonstrated that the efficacy of mucosal elevation produced with the 15 wt % PLGA-PEG-PLGA hydrogel in the colon was excellent and the submucosal layer was almost autodissected due to the administration of hydrogel, as demonstrated in Figure 6.

**Figure 6.** Histological section of porcine colon made immediately after mucosal lifting created with the 15 wt % PLGA-PEG-PLGA hydrogel. A satisfactory SFC, which can lift the mucosa away from the muscularis propria layer, was created. Asterisks indicate the presence of hydrogel that can not be stained by HE. This image was merged from approximate 150 images taken using microscope under 100X magnification.

"Autodissection" Properties. A total of 12 colonic ESDs were performed in 3 pigs and each lesion size was set in appropriate 2 cm. Among them, 10 wt % hydrogels were used in 3 ESDs, 15 wt % hydrogels in 3 ESDs, 20 wt % hydrogels in 3 ESDs, and glycerol fructose as the control in 3 ESDs. As shown in Figure 7, the ESD procedure was significantly simplified with the assistance of thermoreversible hydrogels and repeated injections were not needed. Only one single submucosal injection of 10 mL hydrogel was needed to adequately lift such an assumed 2 cm lesion in 10, 15, and 20 wt % hydrogel groups, whereas repeated injections were required in the glycerol fructose group.

In particular, the thermoreversible hydrogels with high polymer concentrations (15 and 20 wt %) were able to "autodissect" submucosal layer of colon after their submucosal injections, and the intact specimens of mucosa were conveniently achieved and the surface of wounds was clean and tidy, as illustrated in Figure 8. There were no significant complications including major bleeding and perforation during the procedure. A small amount of hydrogel persisting in the wound was found in the hydrogel groups during necropsy. In contrast, there was absence of the "autodissection" function with both the use of 10 wt % hydrogel and glycerol fructose, and further tedious dissection of the submucosal tissue using endoknife was needed, resulting in the rough surface of wounds. The resected specimen in the glycerol fructose group was wrinkling, which was also attributed to the repeated use of endoknife.

Histologic examinations in Figure 9 further confirmed that the intact mucosa specimens were achieved by colonic ESD in all the four groups. No obvious muscularis propria tissues were found in the groups received the administration of thermoreversible hydrogels. In contrast, a small amount of muscularis propria tissue linkage with submucosa was observed in the control group, as shown in Figure 9d, suggesting that the muscularis might be damaged during ESD procedure with the use of glycerol fructose as the submucosal injection agent.

Safety and Efficacy of the 15 wt % Hydrogel As the Optimized System in Both the Nonsurvival and Survival Experiments. Because of the moderate injection pressure, long-lasting mucosal elevation and efficient "autodissection" function, it is obvious that the 15 wt % hydrogel is the optimized hydrogel system for colonic ESD. Table 2 summarizes the results with regard to en bloc resection rate, procedural factors, and complications between 15 wt % hydrogel group and glycerol fructose group in a nonsurvival animal experiment. The en bloc resection rate was 100% in both groups. The mean resected size specimen of hydrogel group was 18.0 ± 3.0 mm, while that of glycerol fructose group was 17.0 ± 3.0 mm (P = 1.00). Both the total operation time and total resection time were similar between the two groups (10.0 ± 2.0 min vs 9.0 ± 4.4 min, P = 0.74; 5.3 ± 1.5 min vs 6.0 ± 5.2 min, P = 0.84). However, repeated submucosal injections were not needed (1.0 ± 0 vs 2.3 ± 0.6, P = 0.02) and injection volume was also smaller in the hydrogel group compared with the glycerol fructose group (10.0 ± 0 mL vs 21.7 ± 2.9 mL, P = 0.00). These results indicate that the use of 15 wt % hydrogel for submucosal injection could simplify colonic ESD procedure, which is very helpful for the operators with low experience. No differences of mean operation time between the two groups may be attributed to the limited sample size and relatively small lesions in this study. Another factor may come from the excellent skills of our operator, Prof. Xu, a famous ESD expert. We believe that the advantage in time of hydrogel should be embodied with larger lesions. Minor bleeding occurred during ESD was successfully managed. No perforation was observed in the hydrogel group, whereas intraoperative perforation occurred in one ESD procedure in the glycerol fructose group. The perforation was then successfully clipped.

Another survival animal experiment was also carried out (data not shown), and no delayed bleeding or perforation occurred.
occurred during follow-up for both the groups. Meanwhile, no other severe complications, such as gastrointestinal tract leak, or secondary abdominal infection, were found during the subsequent necropsy.

**DISCUSSION**

As a minimally invasive treatment, ESD has the ability to endoscopically remove early neoplasms based on the use of electrouactery throughout the gastrointestinal tract.5,6 Nevertheless, this procedure is still not widely adopted in the colon compared with gastric and esophageal ESD due to the greater technical difficulty associated with the anatomical features of the colon, including the longer length, narrow lumen, thinner walls, and extensive flexion, which results in the prolonged procedure time and the increased risk of perforation.1,10,17 To overcome these shortcomings, many new instruments and

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**Figure 7.** Typical images to show the procedure of colonic ESD after submucosal injection of 15 wt % PLGA–PEG–PLGA hydrogel. Endoscopic views of colon mucosa (a) before and (b) after submucosal injection of 10 mL hydrogel; (c) circumferential precutting; (d) “autodissection” during submucosal dissection; (e) taking out of en bloc resected mucosa and (f) the wound left after ESD.

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**Figure 8.** Feasibility of “autodissection” function after submucosal injection during colonic ESD. (a) Different efficacy of “autodissection” of submucosal tissues after submucosal injection of the indicated agents. (b) Completion of en bloc dissection of assumed lesions. (c) Resected mucosal specimens.
devices, such as various cutting knives with different tip configurations, have been developed to make the technique more reliable and thus to prevent complications, particularly perforation. On the other hand, adequate and long-lasting mucosal elevation in the colon by submucosal injection of fluid is also vital to reduce perforation and achieve the en bloc resection of lesions during the colonic ESD procedure. Previously, the available injection solutions were generally designed based on two principles: the osmolarity and viscosity of a solution; however, it is difficult for them to maintain the durable mucosal elevation because of absorption of surrounding tissue. The ideal submucosal injection substance, except for the ability of adequate and long-lasting mucosal elevation, should be low-cost, widely available, conveniently injectable, biodegradable, and biocompatible.

In fact, a few of injectable hydrogels have been tried as the noncolon submucosal injection substances. For example, a photo-cross-linkable chitosan hydrogel was utilized to create the SFCs in esophagus ESD. First, a chitosan solution was submucosally injected and then an in situ hydrogel was obtained via UV irradiating the elevated mucosa for a total of 5 min (30 s each at 10 various sites using an UV lamp system through the endoscopic accessory channel). It is obvious that such a complicated procedure is very inconvenient during its application and the homogeneity of hydrogel cannot be ensured. Also, Khashab et al. have evaluated the potential of another hydrogel developed by Cook Medical in gastric ESD. The composition of this agent was unknown, and such a gel system must equip with a special injection device to deliver this high viscosity gel. Recently, our work has also revealed that thermogelling PEG/polyester copolymers as a novel submucosal injection agent exhibited durable mucosal elevation in the porcine stomach and en bloc resection of lesions was conveniently realized by gastric ESD. To the best of our knowledge, the feasibility and efficacy of these hydrogels as a submucosal injection agent in colonic ESD has not been reported so far.

In the present study, we evaluated the biodegradable and thermoreversible PLGA-PEG-PLGA hydrogel as a submucosal injection fluid in colonic ESD for the first time. It is actually not a gel before administration. As shown in Figure 3, the polymer/NS system is a sol at room temperature and turns into a semisolid hydrogel at body temperature as the polymer concentration is higher than CGC. In contrast, the polymer aqueous solution just exhibits a free-flowing sol state at a broad temperature range (0–50°C) when the polymer concentration is lower than CGC. The thermo-induced gelation of PLGA-PEG-PLGA polymer in water is attributed to the formation of a percolation micellar network upon heating.

In the in vivo study, the colonic mucosa was elevated rapidly after submucosal injection of the PLGA-PEG-PLGA aqueous

<table>
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</table>
solutions with various polymer concentrations and the control group, PLGA-PEG-PLGA hydrogel (Figure 4). The SFCs created with 5 wt % PLGA-PEG-PLGA solution (its polymer concentration is lower than CGC and cannot form hydrogel at body temperature) and glycerol fructose rapidly disappeared within 5 min due to their quick diffusion out of the injection sites. In contrast, the mucosal lifting was prolonged significantly after injection of the PLGA-PEG-PLGA solutions with high polymer concentrations (>CGC). Especially, no obvious changes in size and consistency of the SFCs produced by 15 and 20 wt % PLGA-PEG-PLGA hydrogels was observed over 30 min. Such a lifting efficacy was also remarkably superior to that created by sodium hyaluronate,16 a commonly used submucosal agent. This finding indicates that the formation of in situ hydrogel underneath mucosa is crucial for the maintenance of adequate mucosal elevation, and the hydrogel with higher polymer concentration exhibits higher viscosity (Figure 3b), resulting in the more durable mucosal lifting. The in vivo elimination of such a thermoreversible hydrogel depends on its biodegradation.56,57 Both our previous studies and others have demonstrated that the in vivo degradation of PLGA-PEG-PLGA hydrogels with different MWs or block lengths lasted 4–6 weeks after a subcutaneous injection and the final degradation products were lactic acid, glycolic acid, and PEG, which can be metabolized or cleared in body.40,46,56,57 Meanwhile, the good biocompatibility of PLGA-PEG-PLGA hydrogels has also been confirmed.40,46,56,57

In all cases, the application of Encore 26 inflation device facilitated the submucosal injection of the examined samples, whereas a greater force is required to inject the hydrogel systems compared with glycerol fructose (Figure 5). In particular, it is difficult to achieve a constant flow during injection of 20 wt % PLGA-PEG-PLGA hydrogel through an endoscopic needle. Fortunately, the PLGA−PEG−PLGA hydrogels with 10 and 15 wt % concentrations were easily injectable even if using a conventional 10 mL syringe. One striking advantage of using the thermoreversible PLGA-PEG-PLGA hydrogel for colonic ESD is able to facilitate the whole procedure. The mucosal lifting created by one single injection lasted for the whole ESD procedure, and repeated injections, which will prolong the time of ESD process and increase the work power of operators, were not required. The presence of the hydrogel did not complicate any electrocautery settings, more importantly, the hydrogels with 15 and 20 wt % polymer concentrations exhibited the unique function of “autodissection” of the colonic submucosal layer following their submucosal administration. As shown in Figures 7 and 8, after the circumferential resection of the elevated mucosa, the colonic lesions were neatly dissected en bloc as the result of “autodissection” and further tedious submucosal dissection using endoknife was not needed. It is noteworthy that the commonly used submucosal agents, such as sodium hyaluronate and glycerol fructose, are absent of such a unique “autodissection” function after injection. There was no signs of submucosal or muscularis propria injury. Meanwhile, neither perforation nor major hemorrhage occurred in any experiment with the use of the hydrogels. Though a little amount of hydrogel left in the wound was found during necropsy, the residual hydrogel was biodegradable and biocompatible.40,56–58 What’s more, the intact mucosal samples were obtained via colonic ESD, which are crucial for the accurate pathologic assessment (Figure 9). It is obvious that both the long-lasting mucosal lifting and “autodissection” were attributed to the in situ formation of hydrogel beneath the mucosa after injection. Moreover, the estimated cost of such a hydrogel system is low and do not go over $2/mL according to the experience of our lab. All the features indicate that the thermoreversible PLGA-PEG-PLGA hydrogel has a great potential to promote the technical innovation of colonic ESD if its efficacy and safety are further verified in larger colonic lesions on different animal models and eventual human trials.

**CONCLUSIONS**

The thermoreversible PLGA-PEG-PLGA hydrogel has great potential as an ideal submucosal substance in the colon to achieve high mucosal lifting and long duration and thus to minimize complications associated with colonic ESD. Meanwhile, its unique function of “autodissection” after submucosal injection is meaningful to simplify the colonic ESD technique and make ESD more feasible for large colon lesions that need multiple resections and repeated injections. In addition, considering the injection pressure, duration of mucosal elevation and efficacy of “autodissection”, the 15 wt % hydrogel was the optimized system for colonic ESD.

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**Notes**

The authors declare no competing financial interest.

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