Injectable drug-eluting elastomeric polymer: a novel submucosal injection material

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Background: Biodegradable hydrogels can deliver therapeutic payloads with great potentials in EMR and endoscopic submucosal dissection (ESD) to yield improvements in efficacy and foster mucosal regeneration.

Objective: To assess the efficacy of an injectable drug-eluting elastomeric polymer (iDEEP) as a submucosal injection material.

Design: Comparative study of 3 different solutions by using material characterization tests and ex vivo and in vivo porcine models.

Setting: Academic hospital.

Interventions: Thirty gastric submucosal cushions were achieved with saline solution (0.9%), sodium hyaluronate (0.4%), and iDEEP (n = 10) in ex vivo porcine stomachs. Four porcine gastric submucosal cushions were then created in vivo by using iDEEP.

Main Outcome Measurements: Maximum injection pressure, rebamipide release rate, submucosal elevation duration, and assessment of in vivo efficacy by en bloc resection.

Results: No significant difference in injection pressures between iDEEP (28.9 ± 0.3 psi) and sodium hyaluronate (29.5 ± 0.4 psi, $P > .05$) was observed. iDEEP gels displayed a controlled release of rebamipide up to 2 weeks in vitro. The elevation height of iDEEP (5.7 ± 0.5 mm) was higher than that of saline solution (2.8 ± 0.2 mm, $P < .01$) and sodium hyaluronate (4.2 ± 0.2 mm, $P < .05$). All EMR procedures were successfully performed after injection of iDEEP, and a large gel cushion was noted after the resection procedure.

Limitations: Benchtop, ex vivo, and nonsurvival pig study.

Conclusions: A novel injection solution was evaluated for endoscopic resection. These results suggest that iDEEP may provide a significant step toward the realization of an ideal EMR and endoscopic submucosal dissection injection material.

Abbreviations: ESD, endoscopic submucosal dissection; iDEEP, injectable drug-eluting elastomeric polymer; PEGMC, polyethylene glycol maleate citrate; SH, sodium hyaluronate.

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EMR and endoscopic submucosal dissection (ESD) are minimally invasive procedures to remove early malignant lesions limited to the superficial layers of the GI tract.[1,2] To improve efficacy and safety, EMR and ESD techniques require the injection of a solution underneath the mucosa into the submucosal space.[3] Although numerous injection solutions have been proposed and tested, saline solution or diluted epinephrine with saline solution is the most commonly used in the clinic because of its low cost and ease of use, but it is hampered by rapid dispersion within the submucosal plane, resulting in the need for repeated injections.[4] To improve submucosal lift durations, sodium hyaluronate (SH) is currently being studied because of its high viscosity, ease of injection, and ability to provide long-lasting submucosal lift durations.[5-8] However, high costs and concerns for tumor stimulation limit its widespread use.[3,9]

Recent research indicated a paradigm shift toward the development of mucosal resection injection solutions that rely on gel formation to provide extended submucosal lift durations.[10] Photocrosslinkable chitosan and thermoresponsive poloxamers have been recently reported for EMR with great enthusiasm, but are limited by administration difficulties.[11,12] For example, the liquid-to-gel transformation by using photoinitiated free radical polymerization requires the use of ultraviolet light, which may be difficult in hard-to-reach areas, and thermoresponsive polymers have been shown to clog inside long delivery tools at normal body temperature.[12,13]

We recently reported on the development of biodegradable elastomeric hydrogel, polyethylene glycol maleate citrate (PEGMC), which has been shown to have excellent cyto-/tissue compatibility and controlled degradability both in vitro and in vivo for tissue engineering and drug-delivery applications.[14-16] Although PEGMC not yet evaluated for clinical use, previous studies have shown it to elicit a minimal inflammatory response when injected subcutaneously in rats, with complete material degradation within 4 weeks after implantation. The leachable degradation products of PEGMC hydrogels were evaluated in vitro with NIH 3T3 fibroblasts and were found to be comparable to currently U.S. Food and Drug Administration–approved materials, PEGDA hydrogels.[14] The ability to be injected by using minimally invasive methods and deliver therapeutics in a controlled manner has prompted the investigation of PEGMC as a new EMR injection solution. Unlike previous materials, the liquid-to-gel transformation of PEGMC can provide sustained mucosal lift without administration difficulties, and the controlled release of rebamipide,[17] which stimulates prostaglandin generation and improves the speed of ulcer healing, from the biodegradable gel can potentially aid in mucosal regeneration after resection. The purpose of this study was to evaluate the efficacy and safety of a PEGMC-based iDEEP, addressing the limitations of previous solutions.

Take-home Message

- A novel injectable drug-eluting elastomeric polymer (iDEEP) was developed and evaluated as a submucosal injection material with improved results over the currently available injection solutions.
- iDEEP may provide a significant step toward the realization of an ideal injection material for EMR and endoscopic submucosal dissection.

MATERIALS AND METHODS

All chemicals were purchased from Sigma Aldrich (St. Louis, Mo). PEGMC with different citric acid:maleic anhydride monomer ratios were synthesized as previously described.[14] To prepare the iDEEP part A component (iDEEP-A), PEGMC was dissolved in deionized water (20 wt%), and combined with polyethylene glycol diacrylate (12 wt%), and tetramethylethylenediamine (0.5 wt%). The iDEEP part B component (iDEEP-B) was prepared by dissolving ammonium persulfate redox initiator (0.25 wt%) in deionized water. Combining the part A and B solutions in a 2:1 ratio, respectively, produced iDEEP gels.

To assess the ease of injection, maximum injection pressures were evaluated by using a 25-gauge endoscopic needle (US Endoscopy, Mentor, Ohio), digital manometer (Cole-Parmer, Vernon Hills, Ill), and syringe pump (KD Scientific, Holliston, Mass) connected to a 3-way Luer-lock stopcock delivered at 5 mL/min. To determine the rebamipide release rate, rebamipide (1 mM) was mixed with various iDEEP-A solutions and combined with the iDEEP-B to form cross-linked gels. The drug-loaded gels were then incubated in phosphate-buffered saline solution (37°C; pH 7.4), and rebamipide release was determined by using high-performance liquid chromatography (Waters, Milford, Mass). The upper third of porcine stomachs were used for all ex vivo studies because of the resemblance to the human stomach in thickness and histology. The gastric specimens were obtained immediately after killing, cut into 5 × 5-cm squares, and fixed on a corkboard. Using a 2.5-mL syringe and 25-gauge needle, we injected 1 mL of each solution tangentially into the submucosa through the mucosal surface. Mucosal elevation height was quantitatively determined from photographs by using Image J Analysis software. For the in vivo model, 4 EMR procedures were performed in the stomach of a porcine specimen by using a 25-gauge catheter injection needle. All solutions were mixed with methylene blue (0.5/10 mL of solution) for visualization. An en bloc resection of the elevated mucosa was performed with a hook-knife and polypectomy snare and recorded with endoscopic photographs.

The results are expressed as the mean ± standard deviation (n = 10). The statistical significance between 2

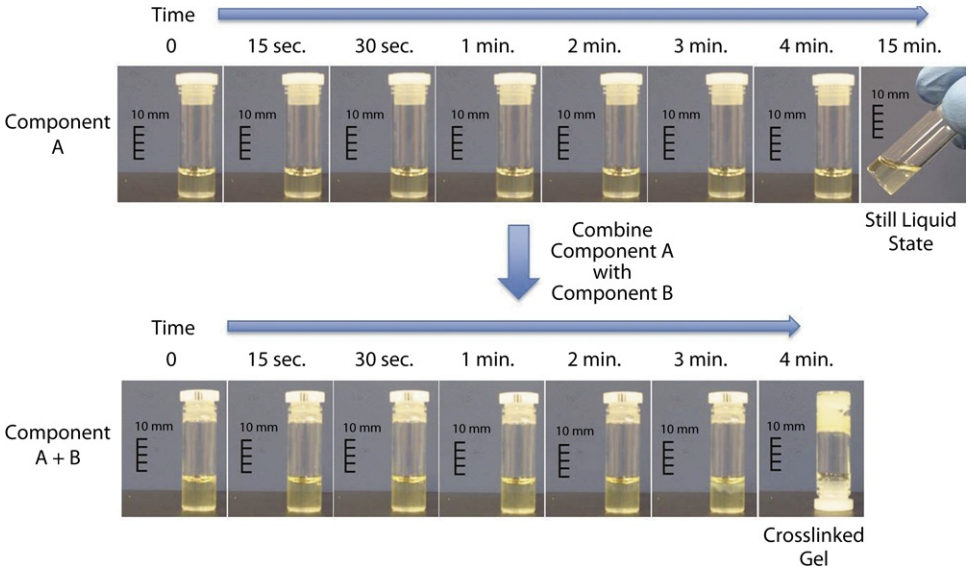

Figure 1. Photographic representation of the liquid-to-gel transformation of the injectable drug-eluting elastomeric polymer. Gel transformation only occurs after the A and B components are combined.

sets of data was calculated by using a 2-tailed Student *t* test, and nonparametric 1-way analysis of variance was performed where appropriate. Data were taken to be significant when a *P* value $<.05$ was obtained.

RESULTS

Liquid-to-gel transformation occurred within 4 minutes of combining the iDEEP part A and B components (Fig. 1). No significant difference in the pressures developed during injection was observed between iDEEP-A (28.9 ± 0.3 psi) and SH (29.5 ± 0.4 psi, $P > .05$). Injection pressures of iDEEP-B were much lower (6.6 ± 0.1 psi) and were found comparable to those of saline solutions (6.0 ± 0.1 psi, $P > .05$). Rebamipide release studies from iDEEP showed an initial burst release at 4 hours for all iDEEP-A compositions. After the initial burst release, a controlled release for up to 2 weeks was observed and could be controlled with the iDEEP-A monomer ratios (Fig. 2). In the ex vivo study (Fig. 3), iDEEP displayed the highest submucosal elevation heights at all time points. After 30 minutes, iDEEP displayed extended lift durations (5.7 ± 0.5 mm) with higher submucosal elevations than those of saline solution (2.8 ± 0.2 mm, $P < .01$) and SH (4.2 ± 0.2 mm, $P < .05$). In the preliminary in vivo study, the iDEEP-A was easily injected in the porcine stomach to create submucosal elevation (Fig. 4A). Using the same injection needle, we injected the iDEEP-B to produce a soft biodegradable gel underneath the mucosa (Fig. 4B). No electrocautery setting changes were needed to perform the procedure.

DISCUSSION

EMR and ESD are minimally invasive endoscopic procedures now accepted worldwide as a treatment modality for the removal of dysplastic and early malignant lesions limited to the superficial layers of the GI tract.[1,2] Unfortunately, the EMR/ESD procedure has been historically limited by the short submucosal lift durations of the available injection solutions, which have been constrained by 2 design avenues: the osmolarity or viscosity of a solution is responsible for the lifting properties of the material.[18] The recent introduction of injectable materials, which use a liquid-to-gel transformation, has shown promise in providing extended submucosal lift durations. However, many of these gel-forming materials are plagued by administration difficulties, which further complicate the procedure. In review of the recent progress in the development of EMR solutions, the ideal injection solution should be cost-effective, widely available, easily injectable, biocompatible, biodegradable, able to provide prolonged submucosal lift durations, and able to aid in mucosal healing after the resection process to have clinical relevance.[4,8]

In this study, we developed a novel iDEEP that aims to meet all of the requirements of an ideal EMR solution and overcome the limitations of previous solutions. As shown in Figure 1, iDEEP uses both viscosity and gel formation through redox-initiated cross-linking to overcome the limitations of previous designs. The water-soluble iDEEP-A, which is more viscous than saline solution, will remain a viscous liquid until combined with the water-soluble iDEEP-B to produce a soft bio-

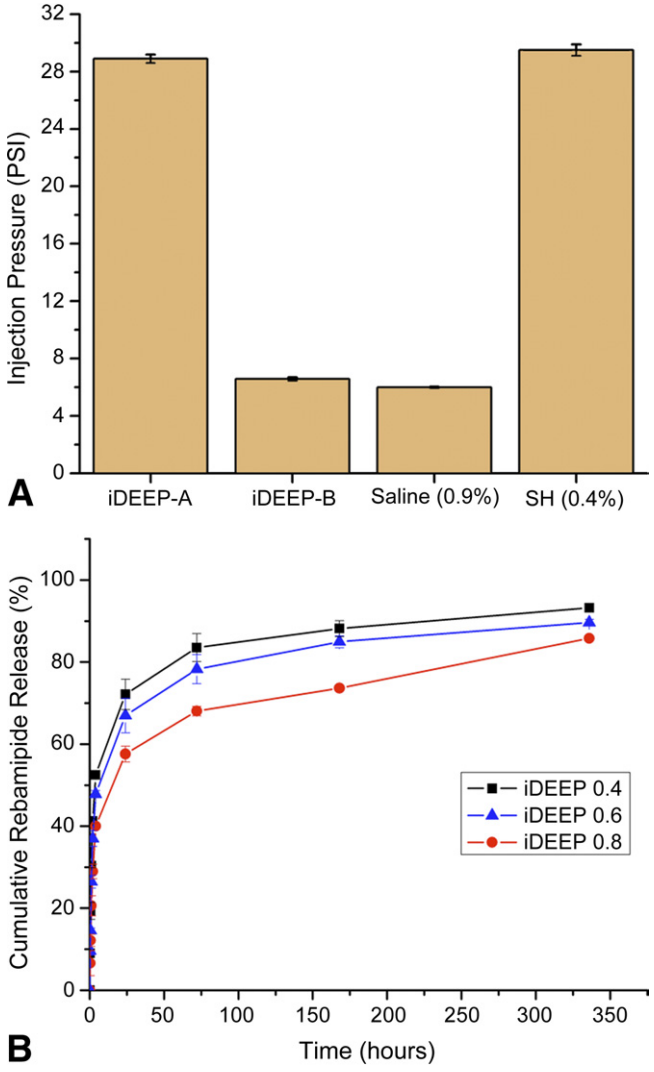

Figure 2. A, Maximum injection pressures of the tested solutions. **B,** In vitro rebamipide release profiles from injectable drug-eluting elastomeric polymer (iDEEP) gels.

degradable hydrogel. Dividing the system into 2 separate components offers a huge advantage over previous designs in that the surgeon can precisely control the gel setting location and time and avoid premature gelling inside the delivery tools. In addition, the use of a redox-initiated cross-linking mechanism does not require the use of additional equipment such as ultraviolet light for gel formation to occur. These criteria have all been developed with the cost-effectiveness of the system in mind. Although the iDEEP system is more expensive than saline solution, it is roughly 33 times less compared with hyaluronic acid solution formulations (Hyalgan, $66/mL) and 2.5 times less compared with a 0.4% hyaluronic acid solution (MucoUp, $5/mL), which is commercially available in Japan.[12,19]

A higher viscosity liquid typically translates into a greater force required to inject the solution through small-caliber delivery tools, which may produce unwanted administration difficulties. To ensure that the iDEEP components were easily injectable, we assessed the maximum pressures developed by using real-life conditions. Injection of the iDEEP-A component through a 25-gauge endoscopic needle had greater difficulty in achieving a constant flow compared with saline solution, but was found comparable to SH. iDEEP-B solutions were even easier to inject, showing injection pressures similar to that of normal saline solution. The localized and controlled delivery of rebamipide,[8] a mucosal protective and ulcer healing drug shown to stimulate prostaglandin generation, may improve the speed of ulcer healing to aid in the management of EMR-induced damage. The in vitro rebamipide release from iDEEP gels displayed an initial burst release followed by a sustained release for as long as 2 weeks and could be controlled through polymer monomer ratios. The developed polymers are also capable of incorporating hemostatic and/or antineoplastic drugs to assist mucosal resection and treatment.

In ex vivo studies, all of the submucosal cushions created with iDEEP were more durable than those performed with saline solution and SH at all time points. No significant changes in iDEEP cushion height were observed after 5 minutes because of gel formation. To minimize any discrepancies and limitations of an ex vivo study, all specimens were obtained within the first hour of the animal's death, and all tests were performed at constant temperature of 37°C to minimize any tissue changes. To evaluate the efficacy of iDEEP, standard EMR procedures were performed in vivo by using a live porcine stomach model. The iDEEP-A was easily injected by using standard delivery tools and was able to create an adequate submucosal cushion. Using the same injection needle, the iDEEP-B solution was then injected into the same location without any clogging inside the delivery tool. After 5 minutes of iDEEP-B injection, the en bloc resection of the elevated mucosa revealed a soft biodegradable gel underneath the mucosa to provide protection for the underlying muscle layer from electrocautery damage. The presence of the iDEEP gel did not complicate the resection procedure or require any changes to the electrocautery settings. Although the iDEEP gel cannot be removed entirely after the EMR procedure, previous studies have shown complete biodegradation of the hydrogel, excellent tissue compatibility, and minimal inflammation.[14] We also believe that the remaining material left behind after the resection procedure can be used to deliver therapeutics and promote regeneration of the damaged mucosa. Although mucosal regeneration was not evaluated in this study, long-term in vivo degradation and mucosal regeneration in porcine stomachs by using survival animal models with detailed pathological review will be the focus of future studies.

In conclusion, iDEEP is a cost-effective, readily available, and easily injectable 2-component solution that

Figure 3. Photographic images depicting the chronological changes in the submucosal elevation of (**A**) saline solution (0.9%) (**B**), sodium hyaluronate (SH) (0.4%), and **C,** injectable drug-eluting elastomeric polymer (iDEEP) (30%) from top to bottom in turn 1, 5, 15, and 30 minutes after injection by using porcine gastric samples ex vivo. **D,** Graph showing the chronological changes in the submucosal elevation.

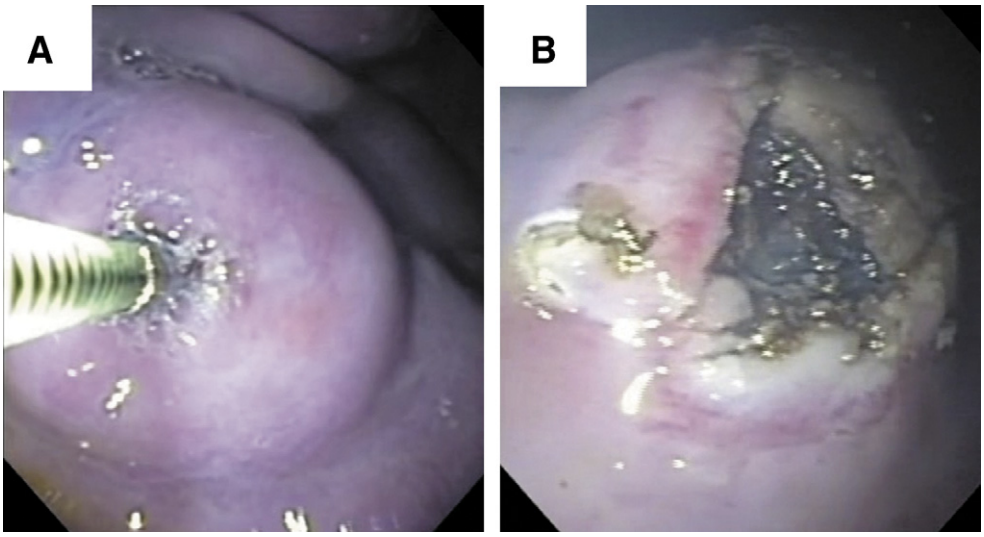

Figure 4. A, Endoscopic views of contained vertical submucosal elevation after injection of the injectable drug-eluting elastomeric polymer (iDEEP) part A and B solution, and **B,** mucosal defect post-iDEEP EMR resection revealing a solidified soft biodegradable gel.

allows biodegradable gel formation under the submucosal space without complex administration difficulties and can potentially aid in mucosal regeneration through controlled therapeutic delivery. iDEEP displayed longer-lasting cushion elevations than other frequently used injection solutions and performed well in EMR procedures in vivo. Although a standard EMR is a relatively quick and easy procedure, our iDEEP solution is potentially very useful in EMR for relatively large lesions that need repeated resections and submucosal injections and for ESD, which is a long-lasting, high-end endoscopic resection technique for GI neoplasms involving a higher risk of perforation.[20] These results suggest that iDEEP may provide a significant step toward the realization of an ideal injection material for EMR and ESD. Although the in vivo resection procedures in this study were only used to determine preliminary efficacy of the iDEEP system, further comparative long-term studies in living animals with pathological review are needed to confirm the efficacy, depth of resection ability, and submucosal regeneration of the iDEEP.

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