

Hemostatic action of EGF-endospray on mucosectomy-induced ulcer bleeding animal models

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Abstract. Gastric bleeding is one of the irritant problems in ulcer patients. In this study, we evaluated hemostatic action of ulcer-coating powder (EGF-endospray) on gastric ulcer animal models. EGF-endospray, containing epidermal growth factor, is designed to be applied through an endoscope. Hemostatic action of the EGF-endospray was evaluated on gastric hemorrhage models of rabbits and micro-pigs. The EGF-endospray was directly applied onto a mucosal resection (MR)-induced gastric bleeding focus in a rabbit model. In a porcine model, the EGF-endospray was applied once via an endoscopy to a bleeding lesion created by endoscopic submucosal dissection. The bleeding focus was then observed via an endoscope. In the rabbit model, EGF-endospray treatment significantly shortened mean bleeding time in comparison with other treatments (104.3 vs 548.0 vs 393.2 s for the EGF-endospray, the non-treated control and the epinephrine injection, respectively). In the micro-pig model, EGF-endospray showed immediate hemostatic action and prolonged covering of the bleeding focus for over 72 h. Histology proved mucosal thickness was more efficiently recovered in all EGF-endospray treated animals. The results of the present study suggest that the EGF-endospray is a promising hemostatic agent for GI bleeding.

Keywords: Gastric bleeding, hemostatic powder, endoscopy, epidermal growth factor

1. Introduction

Gastrointestinal (GI) hemorrhages are a common and significant medical problem that results in hospitalization, morbidity and potentially life-threatening situations [1–3]. GI hemorrhages mostly come from peptic ulcers. Fortunately, the incidence of GI ulcers and ulcer-related hemorrhages consistently decreases by virtue of antibiotic treatment of *Helicobacter pylori* [4]. However, endoscopic mucosal resection (EMR)-induced acute ulcers and hemorrhages become another problematic issues [5,6]. Originally endoscopies were developed for optical diagnosis of GI lesions, but endoscopic treatment of GI

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lesions, that is a form of therapeutic endoscopy, now has major applications (e.g., removal of early gastric cancer, colon polypectomy and other treatments) [7–10]. EMR is now employed rather substantially because it is relatively non-invasive compared to other laparoscopic treatments. However, EMR inevitably induces hemorrhaging which entails proper hemostatic treatments [11].

The most commonly used modalities for hemostatic treatments are epinephrine injection, thermal coagulation using a heater probe, argon plasma coagulation, or mechanical induction of hemostasis using hemoclips [12]. Conventional endoscopic modalities achieve hemostasis in 85–90% of cases when used in combination therapy. However, endoscopic treatment of upper GI hemorrhages can be challenging and requires substantial experience and skill. In addition, despite aggressive proton pump inhibitor treatment, which promotes ulcer healing and reduces rebleeding, bleeding recurs in 15–20% of cases after successful endoscopic hemostasis, mostly within 1 week after primary hemostasis has been achieved [13,14]. Rebleeding can be a serious, life-threatening symptom [15,16]. Therefore, endoscopic treatment of the bleeding focus, which is technically simple to perform during an endoscopic diagnosis, may be more reasonable and effective.

Now a hemostatic spray powder delivered through a scope is under clinical development [17,18]. A catheter is advanced through the scope to within 1–2 cm of the lesion and the powder is sprayed toward the lesion. This technique is so simple and should require a lower level of endoscopic expertise than more conventional therapeutic procedures. In addition, the initial clinical results with the hemostatic powder have been very promising [17]. Among 20 patients with active peptic ulcer bleeding, acute hemostasis was achieved by hemostatic powder delivery in 95% without any major complications. When hemostatic powder comes into contact with moisture at the surface of the lesion, the powder forms a mechanical barrier over the bleeding site and quickly stopping the bleeding. Since the powder coats the ulcer, it is also expected to facilitate ulcer healing as well as hemostasis. However, no basic or clinical research has been conducted to evaluate the ability of the powder to promote ulcer healing.

In the current study, we developed an endoscopic hydrogel powder containing epidermal growth factor (EGF-endospray) which can facilitate hemostasis with additional ulcer healing effects. The EGF-endospray can be applied through an endoscopic catheter during an endoscopy over the bleeding area, form a hydrogel and discontinue local hemorrhaging. Our endoscopic powder contains EGF which can accelerate regeneration of the mucous lesion [19]. EGF promotes cell growth and exerts beneficial effects by either reducing injury or accelerating repair at the wound area. We believe that EGF is released from the ulcer-covering hydrogel, facilitates ulcer-healing and concurrently reduces rebleeding.

Herein we describe an EGF powder (EGF-endospray) that can be directly applied to a gastric bleeding focus via endoscope. First of all, we estimated the hemostatic efficacy of the endoscopic EGF powder against EMR-induced ulcers and bleeding foci of rabbit and porcine models. Ulcer healing efficacy of endoscopic EGF powder was also observed in this study.

2. Materials and methods

2.1. Animals

Six female mini-pigs (Micro-pig[®]; Medi Kinetics, Pyeongteck, South Korea) weighing 35–40 kg, and 46 male rabbits (New Zealand White; Orient Bio, Seongnam, South Korea) weighing 2–2.5 kg were used for our experiments. All animal care and experimental procedures were conducted in accordance with the guidelines of the Experimental Animal Research Committee of Inha University (Incheon, South Korea).

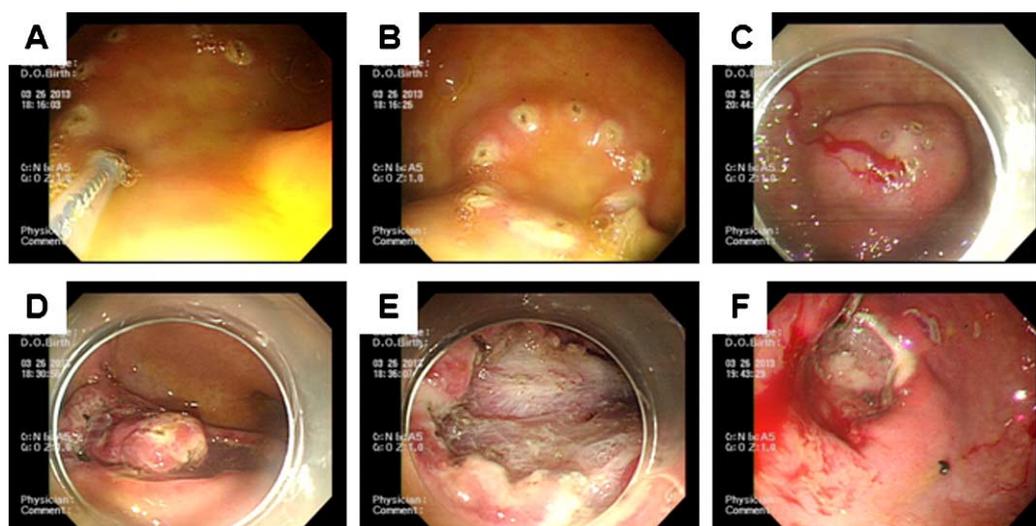


Fig. 1. Endoscopic mucosectomy-induced gastric hemorrhage models of mini-pigs: (A and B) lateral gastric area was marked with an argon plasma coagulator, (C) submucosal injection of isotonic saline, (D and E) endoscopic mucosal dissection was performed, (F) acute ulcers with bleeding. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-141236>.)

2.2. Animal models for gastric hemorrhage

Gastric hemorrhage models were developed against rabbits and micro-pigs in our studies. Rabbit mucosectomy-induced gastric hemorrhage models were developed as follows. Rabbits were fasted for 24 h prior to the operation, then anesthetized with an intramuscular injection of a mixture of ketamine (4.2 mg/kg) and xylazine (11.7 mg/kg). Rabbits were randomly divided into a non-treated group, an epinephrine-injected group and an EGF-endospray treated group. The stomach was exposed and surgically opened along the greater curvature. Then, two hundred microliters of isotonic saline was injected into the submucosal layer of the stomach. The swollen gastric mucosa was resected using a pair of operating scissors. The resected area was around 7–10 mm in diameter. After each treatment, the duration of the bleeding time was measured.

Endoscopic mucosectomy-induced gastric hemorrhage models of minipigs were developed as follows (Fig. 1). First, mini-pigs were deprived of food for 36 h before the endoscopy. Anesthesia was induced via an intramuscular injection of tiletamine/zolazepam (Zoletil[®], Virbac Korea, Seoul, South Korea) and xylazine (Narcoxy-2[®], Intervet Korea Ltd, Seoul, South Korea) and maintained with inhalation of isoflurane (Ifran[®]; Hana Pharm., Kyonggi-do, South Korea) during the operation. The endoscope (GIF-Q260; Olympus Medical Systems, Tokyo, Japan) was inserted with the animals in a lateral decubitus position. The target areas were marked with an argon plasma coagulator (APC), and isotonic saline was injected into the submucosal layer. ESD was performed to produce acute ulcers with diameters of 2.0–3.0 cm in the anterior and posterior walls in the stomach.

2.3. Hemostatic and ulcer-healing action of the EGF-endospray

Hemostatic action of the EGF-endospray on gastric muco-resected rabbit models was estimated. After the laparoscopic resection of the gastric mucosa, the bleeding area was spray-covered with eddo-EGF,

and mean bleeding time was estimated and compared with another conventional treatment; epinephrine local-injection [20].

In the porcine models, muco-resection was performed via an endoscope and then the bleeding-focus was covered with the EGF-endospray. Instant hemostatic action of the EGF-endospray on acute bleeding and rebleeding after the treatment was observed via an endoscopy for 24 h. If bleeding continued within three minutes after first application, a second spray was applied to the wound according to the first application. After the treatment, the animals were observed for 24-hours.

The ulcer healing effect of the EGF-endospray was mainly observed via the assessment of histology. One day after the treatments, the stomach was recovered and the related ulcer area was calculated as the ulcer area divided by the initial ulcer area. For the porcine models, ulcers were observed via endoscopies. Three days after the treatments, the mini-pigs were killed and tissues were harvested and stained for histological assessment.

2.4. Histological studies

The recovered mucosal tissues were fixed in 4% formalin, embedded in paraffin blocks and then stained with H&E staining using routine procedures. H&E-stained sections were analyzed to assess gastric glandular structures and measure the thickness of the regenerated gastric mucosa at the ulcer site under a microscope.

2.5. Statistical analysis

All data are reported as the mean \pm SEM. Experimental results were analyzed with a *t*-test, a one-way ANOVA on ranks followed by Tukey's post-hoc test or a two-way ANOVA on ranks followed by Bonferroni multiple comparisons using PRISM 5 software. *p*-values <0.05, <0.01, <0.001 or <0.0001 were considered statistically significant.

3. Results

3.1. Hemostatic function of EGF-endospray

The hemostatic function of the EGF-endospray was observed on gastric muco-resection-induced bleedings of rabbits and micro-pigs. In the rabbit models, the average bleeding time under each treatment is shown in Fig. 2. Non-treated and epinephrine local-injection yielded around 548.0 s and 39.22 s of bleeding time, respectively. Contrarily, the EGF-endospray displayed a significantly shortened bleeding time (104 s, $p < 0.001$). In the micro-pig models, EGF-endospray also showed immediate hemostatic function, covering the bleeding focus (Fig. 3). Bleeding stopped in 12 min to 26 min and was secured for 72 h after the 1st treatment of the EGF-endospray (Fig. 4). No secondary treatment was required in this study.

3.2. Ulcer healing activities of the EGF-endospray

After allowing 1 day of healing, rabbits were killed with an intramuscular injection of ketamine/xylazine (10/3 mg/kg). Gastric tissues were harvested and further processed for histology. Direction visualization of the gastric mucosa revealed the ulcer healing was precipitated with the EGF-endospray treatment. The relative ulcer size was significantly reduced in comparison with the non-treated

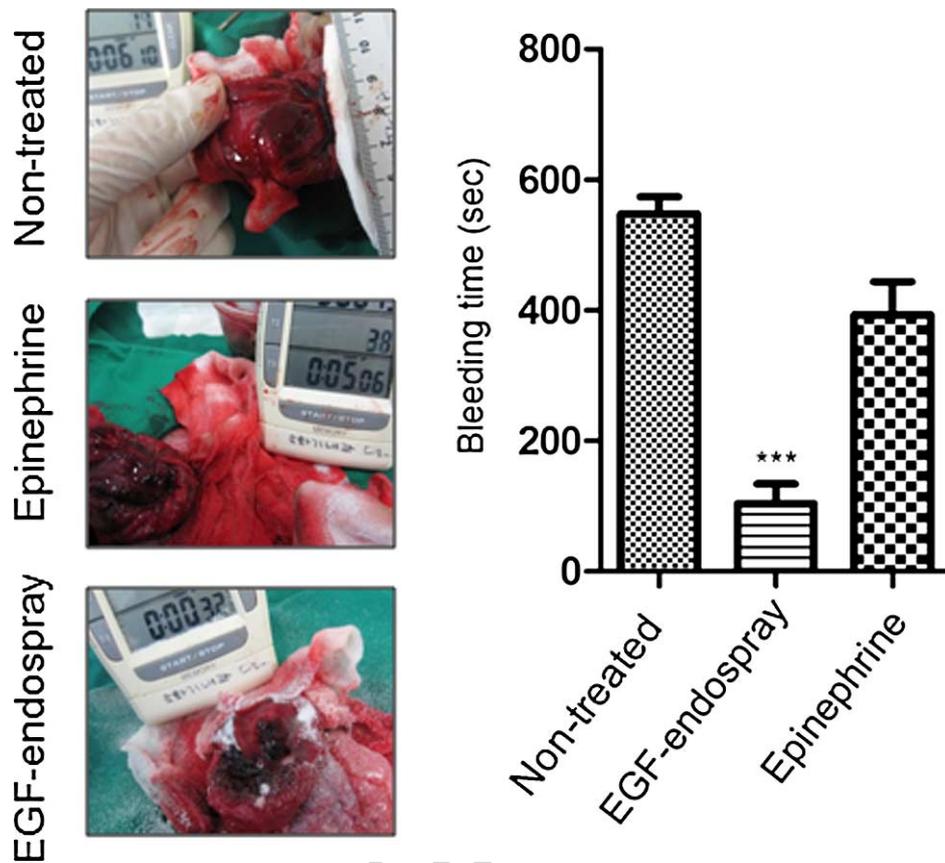


Fig. 2. Hemostatic action of EGF-endospray on the mucosal resection-induced gastric bleeding model of rabbits. The EGF-endospray statistically shortened the mean gastric bleeding time in comparison with the non-treated and epinephrine local injection groups (** $p < 0.001$). (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-141236>.)

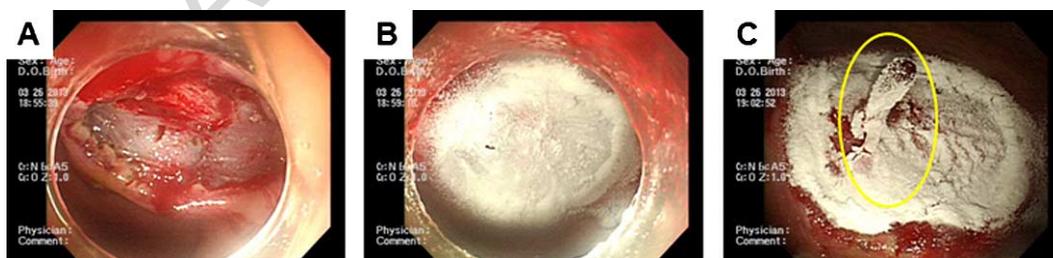


Fig. 3. Hemostatic action of the EGF-endospray on the endoscopic mucosal resection-induced gastric bleeding model of minipigs: (A) gastric hemorrhage before treatment, (B) endoscopy image of gastric ulcer at 3 min after EGF-endospray treatment, (C) endoscopic image of gastric ulcer at 6 min after EGF-endospray treatment. EGF-endospray effectively covered bleeding lesion and performed hemostatic action. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-141236>.)

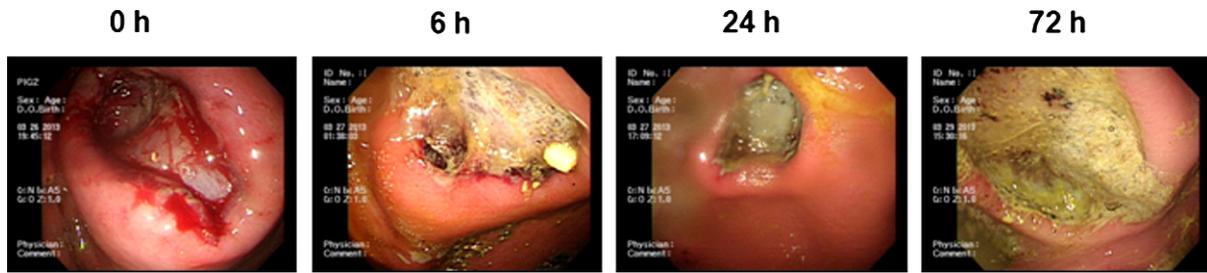


Fig. 4. Endoscopic observation of the extended ulcer covering and hemostatic action of the EGF-endospray up to 72 h on gastric hemorrhage models of micropigs. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-141236>.)

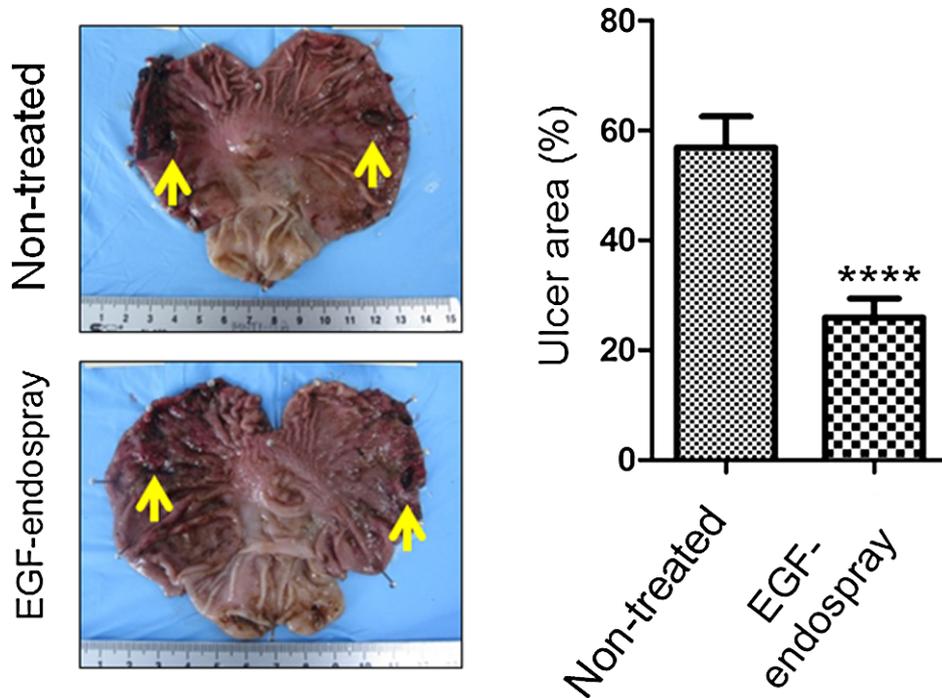


Fig. 5. Efficacy of EGF-endospray for ulcer healing in rabbit mucosal resection-induced gastric ulcer model. Ulcer area (%) was acquired by dividing initial ulcer size by the healed ulcer size. **** $p < 0.0001$. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-141236>.)

groups (Fig. 5). Histology also supported the EGF-endospray precipitated recovery of the gastric mucosa structure (Fig. 6). The basal thickness of mucosa after the resection showed $117 \pm 12 \mu\text{m}$ and then recovered to $389 \pm 37 \mu\text{m}$. In previous our study, chitosan hydrogel with EGF showed more precipitated recovery than EGF-endospray in rabbit gastric ulcer model [21]. We assumed that chitosan hydrogel is more effective in ulcer recovery than EGF-endospray powder. But in aspect of gastric bleeding-control, EGF-endospray might be more effective than chitosan hydrogel.

EMR-induced ulcers in the mini-pigs also displayed enhanced ulcer-healing and rehabilitated mucosa structure (Fig. 7).

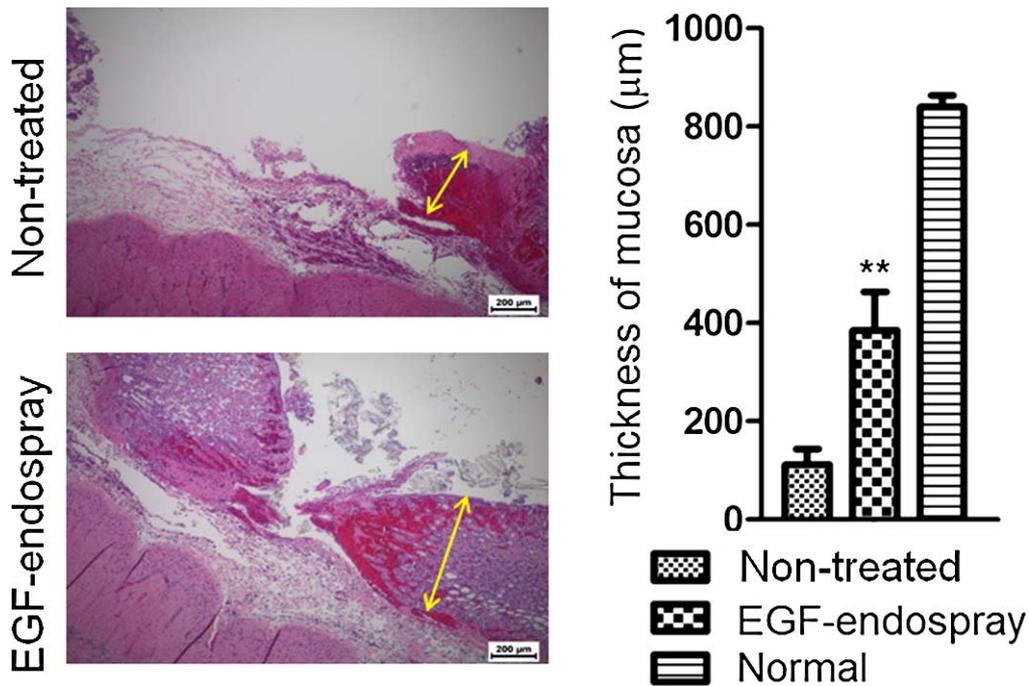


Fig. 6. Histological observation of precipitated mucosal healing (thickness) after treatment of EGF-endospray on rabbit gastric ulcer and hemorrhage models (** $p < 0.01$). (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-141236>.)



Fig. 7. H&E staining images of control (non-treated) and EGF-endospray treated mucosa in mini-pigs. The EGF-endospray forms a covering hydrogel on the ulcer lesion (scale bar represents 200 µm). (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-141236>.)

4. Discussion

In the United States, the annual incidence of upper GI hemorrhaging is around 50–150 per 100,000 people. Every year more than 300,000 people are hospitalized and about 30,000 people die from GI hemorrhages [4]. An estimated annual cost for GI hemorrhage is almost \$1.0 billion. The best way for the diagnosis of GI hemorrhages is via an endoscopy. However, endoscopic modalities for hemostatic treatment are very confined. Epinephrine local injection has been introduced as an endoscopic treatment for GI hemorrhages. Hemoclips, thermal coagulation and argon plasma coagulation are choices of treat-

ments [22]. However these methods still possess technical issues. Positioning the endoscopic devices right on the lesser curve of the stomach and posterior wall of duodenal bulb needs technical expertise and experience. Sometimes coagulation induces undesired mucosal damage and causes late re-bleeding. An ulcer-covering gel works as a mechanical barrier against corrosive gastric juices and may reduce the risk of late re-bleeding [21,23].

In this study, we evaluated the hemostatic and ulcer-healing effects of an EGF-endospray. Our EGF-endospray, contains highly absorptive polysaccharides, and can be applied to GI bleeding foci via an endoscope. Absorptive polysaccharides were selected based on the previous reports (hydroxypropyl methyl cellulose, hydroxyethyl cellulose and alginate) [24,25]. When in contact with blood/gastric fluids, the EGF-endospray absorbs moisture and forms a bioadhesive hydrogel which acts as a mechanical barrier and precipitates ulcer-healing [1,23]. Polysaccharide actively absorbs the fluid around the bleeding lesion, concentrates clotting factors (platelets, thrombin and fibrin) and induces the quick formation of a blood clot. The hydrogel not only acts as a hemostatic agent but also protects bleeding ulcers from corrosive gastric juices and releases EGF, which functionally triggers cell proliferation and mucosa rehabilitation [19,26]. The covering hydrogel is sustained for one or two days after the application, subsequently dissolves out into lumen and finally completely eliminates from the GI tract.

5. Conclusion

In conclusion, we observed the hemostatic action of an EGF-endospray on GI bleeding in animal models. The EGF-endospray could be new therapeutic endoscopic-based treatment for GI ulcer bleedings.

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