Effect of Carboxymethyl Cellulose Sheets in Preventing Esophageal Stricture After Near Circumferential Endoscopic Submucosal Dissection in a Porcine model

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Effect of Carboxymethyl Cellulose Sheets in Preventing Esophageal Stricture After Near Circumferential Endoscopic Submucosal Dissection in a Porcine model

INTRODECTION: Esophageal stricture is a major complication after near circumferential endoscopic submucosal dissection (ESD). We aimed to investigate the effect of carboxymethyl cellulose (CMC) sheets on preventing post-ESD esophageal stricture in a porcine model.

METHODS: Sixteen porcine models were randomly assigned to the CMC or control group after undergoing 5-cm-long circumferential esophageal ESD. In the CMC group, the mucosal defect was fully covered by CMC sheets immediately after ESD, whereas the control group underwent circumferential ESD only. Endoscopy and functional lumen imaging probe (EndoFLIP®) were performed at baseline, 1, 2, and 4 weeks after ESD. After sacrificing the pigs at 4 weeks, macroscopic and histopathological evaluations were conducted.

RESULTS: The stricture ratio (%) using the formula $[1- (D_{max}/50\% (D_{PD} + D_{DD}))] \times 100\%$ showed that the CMC group had a significantly lower stricture ratio compared to the control group (53.5% vs 69.2%, $p<0.001$). In the EndoFLIP® evaluation at 4 weeks, the CMC group exhibited significantly higher distensibility index compared to the control group (0.30 mm^2/mmHg vs 0.18 mm^2/mmHg, $p=0.005$). Histopathologically, the CMC group exhibited a significantly higher re-epithelialization ratio (0.54 vs 0.34, $p=0.015$) and significantly lower levels of inflammation cell counts (346.2 vs 486.0, $p=0.001$) and fibrosis thickness (1841.8 μm vs 3392.3 μm, $p=0.015$) compared to the control group.

CONCLUSION: We found that CMC sheets have a potentially more advantageous effect in preserving esophageal physiological function during post-ESD wound recovery. The use of CMC sheets may be an effective strategy to prevent esophageal stricture after near circumferential ESD.

Key words : Esophageal Stricture, Endoscopic Submucosal Dissection, Carboxymethyl Cellulose, Prevention

I. INTRODUCTION

Endoscopic mucosal dissection (ESD) is an endoscopic tumor resection method that allows for complete removal of large lesions and accurate histopathological examination. [1] Based on long-term data showing comparable outcomes to surgery for superficial esophageal squamous cell carcinoma with low or no risk of lymph node metastasis, ESD has been accepted as a feasible alternative to surgical resection in appropriate cases. [2] ESD related complications include bleeding, perforation, [3] and near circumferential esophageal ESD, in particular, can lead to post-procedure stricture. The risk of esophageal stricture has been reported to increase in patients with mucosal defects involving more than 75% of the esophageal circumference. [4] Post-ESD esophageal strictures can cause dysphagia, severely reduce quality of life, and require multiple endoscopic dilations in patients. Therefore, preventing post-ESD esophageal stricture is crucial, and most clinical practice guidelines for esophageal ESD recommend prophylactic treatment to prevent post-ESD esophageal strictures. [5-7] Various strategies have been explored to prevent post-ESD esophageal strictures, including endoscopic balloon dilation, [8, 9] placement of self-expandable metal stents, [10, 11] oral steroid therapy, [12-14] and local injection of corticosteroids. [14-16] However, these treatments are not fully effective and can carry a risk of adverse events.

Carboxymethyl cellulose (CMC) sheet is a biodegradable material made from oxidized regenerated cellulose, derived from vegetable cellulose. Upon contact with blood or moisture, it forms a transparent gel that adheres to tissues, preventing adjacent tissue surfaces from adhering to each other. This adhesive gel acts as a barrier, protecting tissues from inflammatory mediators and bacteria, while maintaining a hypoxic, moist environment that supports wound healing. CMC sheets are widely used in abdominal and gynecological surgeries to prevent postoperative adhesions, and it is believed they may also be effective in preventing post ESD strictures by inhibiting scar formation during mucosal wound healing.

This study aimed to investigate the efficacy of CMC sheets in preventing post-ESD esophageal stricture, focusing on structural and functional recovery, including the preservation of peristaltic function as measured by endoluminal functional lumen imaging probe (EndoFLIP®).

2. METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee (Seoul, Korea) and Yonsei University. Sixteen 24-week-old pigs, weighing between 30 kg and 35 kg (Micro-pig; Medi Kinetics, Pyeongtaek, Korea), were randomly

divided into the CMC group (n = 8) and control groups (n = 8). The CMC group underwent ESD with the immediate attachment of a CMC sheet, while the control group underwent ESD without sheet attachment. During the 4 weeks following the procedure, the degree of esophageal stricture was evaluated in both groups.

2.1. Preoperative preparation

The pigs were checked daily for 7 days before the ESD procedure. The animals underwent a 24-hour fast and were premedicated with intramuscular atropine sulfate (0.05mg/kg), alfaxane (1mg/kg) and xylazine (2mg/kg) for anesthesia induction. After endotracheal intubation, inhalation anesthesia was maintained with 2% isoflurane. All procedures were performed under continuous cardiorespiratory monitoring.

2.2. EndoFLIP® procedure

The EndoFLIP® measurements were taken prior to the ESD procedure. After anesthesia, an upper gastrointestinal endoscope (GIF-Q260J; Olympus, Tokyo, Japan) was inserted, and the EndoFLIP® probe was placed in the esophagus at 30–35 cm from the upper incisor. Once the catheter is in position, the balloon is distended stepwise in 10 mL increments up to 40 mL.

The minimal diameter (D_{min}) (mm) and distensibility index (DI) (mm^2/mmHg) were measured using the EndoFLIP□ catheter filled with saline at volumes of 30 and 40 mL. Cross-sectional area (CSA) and the corresponding distention pressure were also measured at the same volumes. EndoFLIP□ measurements were monitored in real-time to confirm proper bag placement through direct visualization via the endoscope and the CSA data displayed on the recording unit. If the bag migrated or was interrupted by esophageal peristalsis, it was repositioned, and the measurement was repeated. After the measurements were taken, the balloon was deflated and the catheter was removed.

2.3. ESD procedure

After measuring EndoFLIP® values, the probe was removed, and ESD was performed at the measurement site using an endoscope with a transparent cap (ND-201-1802; Olympus). The circumferential area was marked on both the cranial and caudal sides by argon plasma coagulation (APC 300; Erbe, Tübingen, Germany), and submucosal dissection was carried out with the combined use of Dual knife and IT-2 knife (KD-611L; Olympus) after submucosal injection of saline. To induce stenosis, the mucosal defect was intentionally created to be larger than 5 cm in the longitudinal axis and more than 75% of the circumference in the transverse axis (**Figure 1**). Once the ESD procedure was complete, the size and location of the mucosal defect were recorded.

2.4. CMC sheet treatment

The CMC sheets were prepared by cutting them into small pieces measuring

approximately 50 x 50 mm each, and they were stored in moisture-proof conditions. In the CMC group, the CMC sheet was attached immediately to the mucosal defect area after the completion of ESD. To accommodate the CMC sheets, the transparent cap was switched to a longer and broader distal tip (MH-463/MH-594; Olympus). A piece of CMC sheet (50 x 50 mm) was grasped with biopsy forceps and pulled back into the cap. Once the sheet was positioned above the mucosal defect, it was deployed onto the surface by releasing the biopsy forceps. This procedure was repeated until the mucosal defect was fully covered by the sheets. In the control group, the step of attaching the sheet was omitted, and no additional intervention was performed in the mucosal defect area (**Figure 1**).

2.5. Post ESD care

All pigs were maintained on complete fasting for 24 hours, followed by a liquid diet on second day after ESD. A soft diet was given subsequently. After 2 days from the ESD, a standard diet was provided and continued. Daily food intake, weight changes, and instances of regurgitation and vomiting were monitored. Scheduled postoperative endoscopies were performed on days 7, 14, and 28 after ESD. After the fourth endoscopic examination on day 28, all pigs were euthanized by administrating potassium chloride (1-2mmol/kg) under anesthesia, and the esophagus of each pig was harvested for macroscopic and histological evaluations.

2.6. Evaluation for esophageal stricture

2.6.1. General condition and endoscopic examination

Weight change was measured by comparing the body weight immediately before ESD and body weight at 4 weeks after ESD. The degree of dysphagia experienced by each pig was scored using a standard five-point scale: grade 0 (no dysphagia), grade 1 (some solid food), grade 2 (liquids only), grade 3 (difficulty with liquids and saliva), and grade 4 (complete dysphagia). [17] We checked the mucosal defect area and the ability of a 9.9-mm-diameter endoscope to pass through the esophageal lumen at 1, 2, and 4 weeks after ESD to assess post-ESD stricture.

2.6.2. Assessment of stricture ratio

After the resected esophagus was longitudinally incised and fixed on a foam board, the degree of esophageal mucosal stricture was evaluated using the following formula: Stricture ratio (%) = [1− (D_{max}/50% (D_{PD} + D_{DD}))] x 100% (**Figure 2**). D_{max} represents the length of the short axis at the site of the narrowest regenerative mucosa, while D_{PD} and D_{DD} represent the lengths of the short axis at the normal mucosa on the proximal and distal edges located 3 cm from the narrowest stricture site (D_{max}), respectively.

2.6.3. Assessment of EndoFLIP® data

The D_{min} (mm) and DI (mm^2/mmHg) were measured before ESD and at 1, 2, and 4 weeks

after ESD at the site where ESD was performed.

2.6.4. Histological evaluation

After macroscopic evaluation, the esophageal specimens were examined following staining with hematoxylin-eosin (H&E), Masson trichrome, anti-myeloperoxidase (MPO) antibody, and α-smooth muscle actin (α-SMA). The extent of epithelial coverage, degree of inflammation, and fibrosis were evaluated.

Relative wound re-epithelization was calculated using the formula (MD − NE)/MD, where MD represents the mucosal dissection diameter (μm), measured in a cross-section of the fixed sample at 4 weeks, and NE represents the diameter at the maximal length of the long axis for the non-epithelialized tissue. The border between the re-epithelization and original mucosal epithelium was identified by the absence of any muscularis mucosal tissues (**Figure 3**). The degree of inflammation was assessed through a semi-quantitative analysis of inflammatory cells. A blinded investigator counted stained cells between the epithelium and the muscular layer in each of 5 randomly selected high-power fields (HPF) at ×400 magnification in MPO staining. The fibrosis thickness of the submucosal layer was measured as the maximum thickness of the submucosal fibrotic tissue in Masson's trichrome staining in the vertical direction. The abundance of α-SMA-positive myofibroblasts was also evaluated using special staining [18].

2.7. Statistical analysis

Continuous variables are expressed as means ± standard deviations, while categorical variables are expressed as counts and percentages. The Student t-test or Mann–Whitney U-test was used to compare differences in continuous variables between the CMC group and the control group. Categorical variables were compared by the chi-square test or Fisher’s exact test. All p-values were two-sided, and a p-value < 0.05 was considered statistically significant. Statistical analysis was carried out using SPSS 19.0 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY, USA).

3. RESULTS

3.1. Comparison of general condition and endoscopic examination

Procedure results are presented in **Table 1**. ESD was performed successfully in both groups without any complications, including perforation or bleeding. The size of the resected specimen (cm), calculated by multiplying the lengths of the long axis and short axis, showed no significant difference between the two groups.

The weight change ratio between baseline and week 4 did not show a significant difference between the CMC group and the control group (**Table 1**). Out of a total of 16 pigs, 6 in the control group and 5 in the CMC group exhibited dysphagia. In week 4,

dysphagia at severity levels ≥2 was observed in 3 pigs in the control group and 1 pig in the CMC group. However, no statistically significant difference was found (**Table 1**).

On endoscopic examination, the CMC group showed mild stricture and better patency of the esophageal lumen compared to the control group (**Figure 1**). Although there was no statistically significant difference, the CMC group had more successful endoscopic passage compared to the control group (**Table 1**). Narrowing with a pinhole appearance was observed in three pigs in the control group (one pig in the second week and two pigs in the fourth week). In contrast, only one pig in the CMC group showed pinhole narrowing in the fourth week.

Table 1. Comparison of clinicopathologic data between the CMC group and the control group

	Control	**CMC**	**P value**
Procedure results			
ESD procedure time (min)	31.4 ± 4.0	30.5 ± 5.3	0.721
En bloc resection	5 (62.5)	6 (75.0)	1.000
Size of the resected specimen (cm)	26.6 ± 2.0	27.7 ± 1.4	0.234
Adverse events	0	0	–
General condition			
Weight change ratio	1.08 ± 0.16	1.05 ± 0.11	0.686
Occurrence of dysphagia	6 (75.0)	5 (62.5)	1.000
Degree of dysphagia ≥2	3 (37.5)	1 (12.5)	0.569
Endoscopic examination			
Endoscopic passage	5 (62.5)	7 (87.5.)	0.569
Macroscopic evaluation			
D_{max} (mm)§	9.38 ± 1.19	14.63 ± 2.77	0.001
Stricture ratio (%)	69.21 ± 3.43	53.5 ± 7.11	<0.001
Pathologic evaluation			
Mucosal dissection diameter (μm)	38380.5 ± 6120.2	40141.9 ± 4336.3	0.878
Re-epithelization ratio	0.342 ± 0.136	0.541 ± 0.117	0.015

Inflammatory cell counts, HPF	486 ± 82.0	346.2 ± 48.4	0.001
Fibrosis thickness (μm)	3392.3 ± 967.3	1841.8 ± 1154.9	0.015
Thickness of myofibroblast bundles (μm)	371.5 ± 87.5	242.1 ± 85.0	0.010

CMC, carboxymethyl cellulose; HPF, high-power fields
Values are expressed as mean ± standard deviation or number of cases with percentages in parentheses.
§: Diameter at maximal stricture site

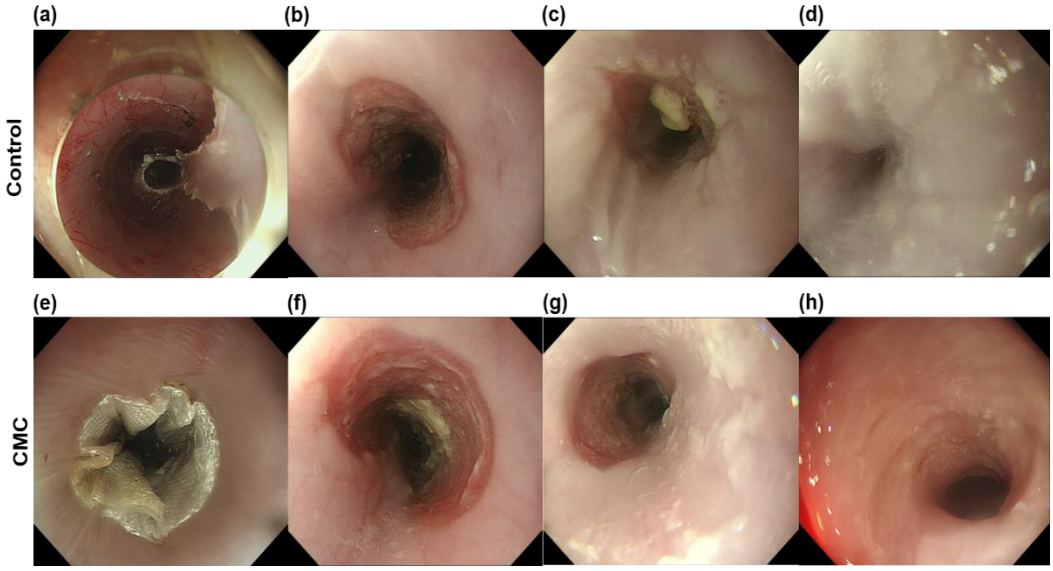

Figure 1. Comparison between the carboxymethyl cellulose (CMC) group and the control group under endoscopic examination over a time course. (a, e) Endoscopic appearance immediately after endoscopic submucosal dissection, showing full coverage of CMC sheets on the mucosal defects in the CMC group. (b, f) Endoscopic appearance at week 1, with the control group exhibiting delayed coverage by granulation tissue. (c, g) Endoscopic appearance at week 2, with the control group showing more stricture progression compared to the CMC group. (d, h) At week 4, pinhole stricture was observed in the control group.

3.2. Esophageal stricture ratio

After sacrificing the pigs on day 28, esophageal patency was evaluated under macroscopic examination. The harvested esophageal specimens were incised longitudinally and fixed on a foam board, and the esophageal mucosal stricture ratio was calculated using the formula mentioned above (**Figure 2**). The CMC group had a significantly higher diameter

at the maximal stricture site and a significantly lower stricture ratio than the control group (**Table 1**).

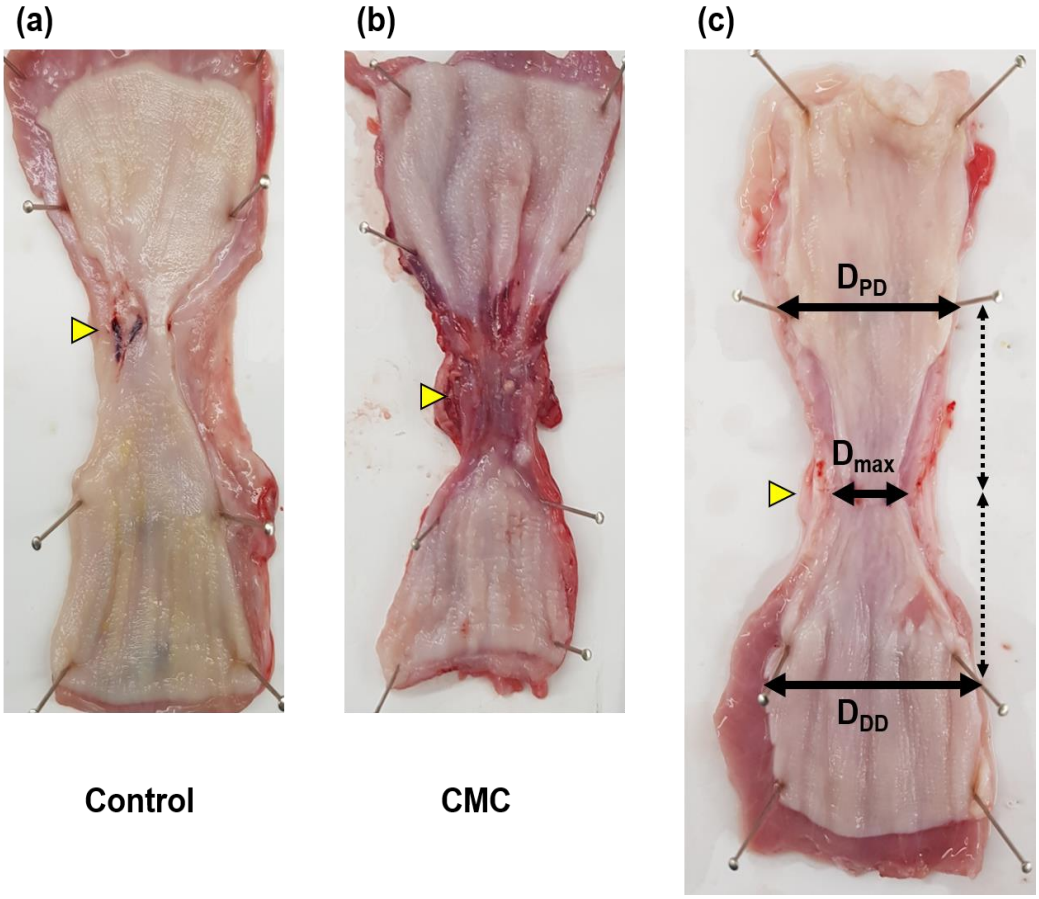

Figure 2. Macroscopic findings of the esophageal specimens. The yellow arrowhead indicates the D_{max} of each specimen. (a) The control group exhibited severe stricture with scar formation. (b) The carboxymethyl cellulose group displayed mild stricture with less scarring of the esophageal mucosa. (c) The patency ratio was calculated as the ratio of the narrowest diameter of the regenerated mucosa (D_{max}) to the average short-axis length of the proximal diameter (D_{PD}) and distal diameter (D_{DD}), measured 3 cm away from D_{max}.

3.3. EndoFLIP® data

The D_{min} values showed no significant differences between the two groups at baseline, week 1, and week 4 (**Table 2**). However, at week 2, the CMC group exhibited significantly higher values in both the 30 mL and 40 mL volumes compared to the control group. The DI values showed no significant differences between the two groups at baseline, week 1,

and week 2 (**Table 2**). However, at week 4, the CMC group showed significantly higher values in both the 30 mL and 40 mL volumes compared to the control group.

Table 2. Comparison of EndoFLIP® data between the CMC group and the control group

	Control	**CMC**	**P value**		**Control**	**CMC**	**P value**
D_{min}, 30 ml				D_{min}, 40 ml			
Baseline	14.24 ± 1.20	15.14 ± 1.25	0.234	Baseline	15.78 ± 1.29	16.70 ± 1.61	0.234
1 week	10.55 ± 1.76	11.16 ± 2.08	0.574	1 week	11.53 ± 1.91	11.86 ± 2.80	0.645
2 weeks	6.20 ± 0.89	8.14 ± 1.63	0.015	2 weeks	6.44 ± 0.85	8.78 ± 2.13	0.021
4 weeks	5.18 ± 0.24	5.79 ± 0.76	0.130	4 weeks	5.45 ± 0.60	6.06 ± 1.10	0.382
DI, 30 ml				DI, 40 ml			
Baseline	2.26 ± 0.88	2.33 ± 0.88	0.878	Baseline	1.71 ± 0.78	1.74 ± 0.80	0.878
1 week	1.00 ± 0.51	0.95 ± 0.68	0.505	1 week	1.13 ± 0.70	0.75 ± 0.37	0.279
2 weeks	0.49 ± 0.27	0.63 ± 0.44	0.574	2 weeks	0.31 ± 0.06	0.44 ± 0.26	0.218
4 weeks	0.30 ± 0.19	0.48 ± 0.22	0.050	4 weeks	0.18 ± 0.05	0.30 ± 0.09	0.004

EndoFLIP, endoluminal functional lumen imaging probe; CMC, carboxymethyl cellulose; D_{min}, minimal diameter; DI, Distensibility Index (mm2/mmHg).
Values are expressed as mean ± standard deviation

3.4. Pathologic findings

Although there were no differences in mucosal dissection diameter (μm) between the two groups, the re-epithelization ratio was significantly higher in the CMC group compared to the control group (**Table 1**). In MPO staining, the control group showed massive infiltration of inflammatory cells on the erosive surface. In contrast, the CMC group exhibited localized and mild infiltration of inflammatory cells in scattered areas (**Figure 4**). In the calculation of the number of inflammatory cells, the CMC group showed significantly fewer cells per HPF at the ESD wound site compared to the control (**Table 1**). In Masson's

trichrome staining, the CMC group showed significantly thinner fibrosis thickness compared to the control group (**Figure 4, Table 1**). In α-SMA staining, the α-SMA-positive cell bundles within the fibrous tissue were significantly thinner in the CMC group compared to the control group (**Figure 4, Table 1**).

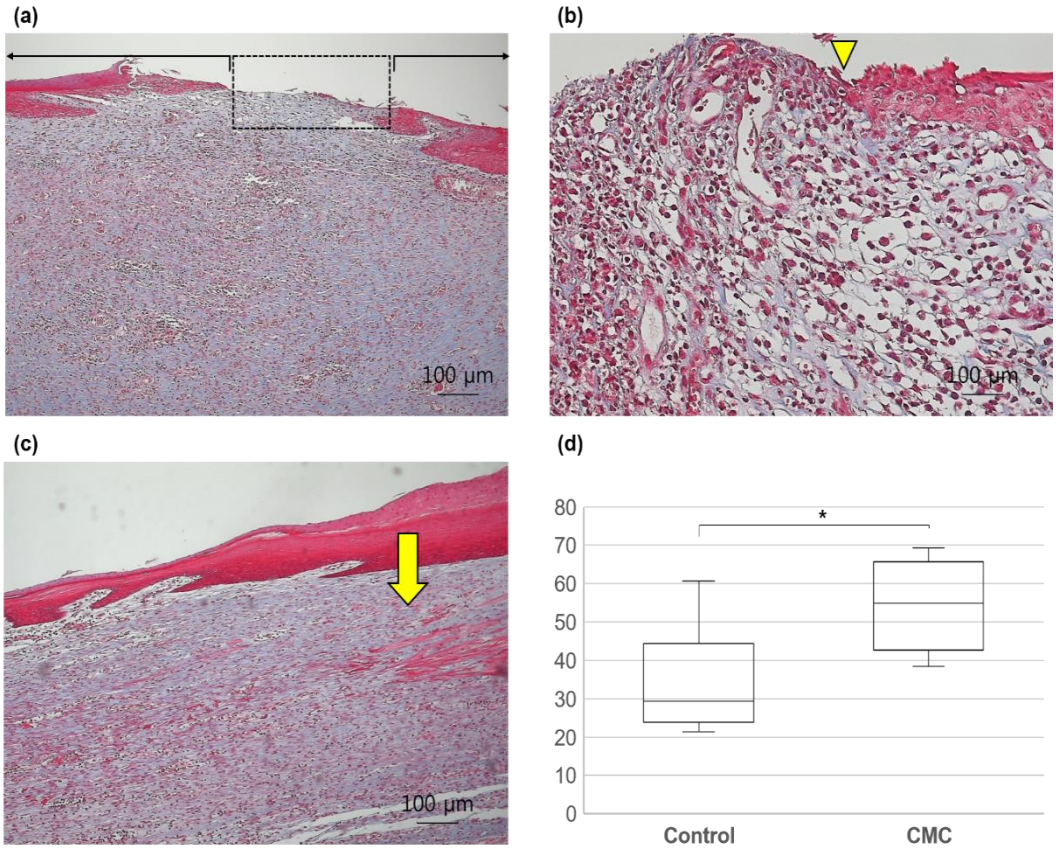

Figure 3. Histologic evaluation of the degree of wound re-epithelialization. (a) The dashed box indicates the non-epithelialized area, while the solid arrowed line indicates the epithelialized area of the wound (Masson trichrome, x 40). (b) A magnified image of the boxed region shows the regenerated squamous layer extending from the right side of the image to the left without an epithelial process. The yellow arrowhead indicates the edge of the regenerated squamous layer (Masson trichrome, x 200). (c) The border of mucosal dissection diameter identified by the absence of muscularis mucosa in the original mucosa (yellow arrow) (Masson trichrome, x 40). (d) Percentage of re-epithelialization in control (n = 8) and the carboxymethyl cellulose wounds (n = 8) at 4 weeks. *P value ≤ 0.05.

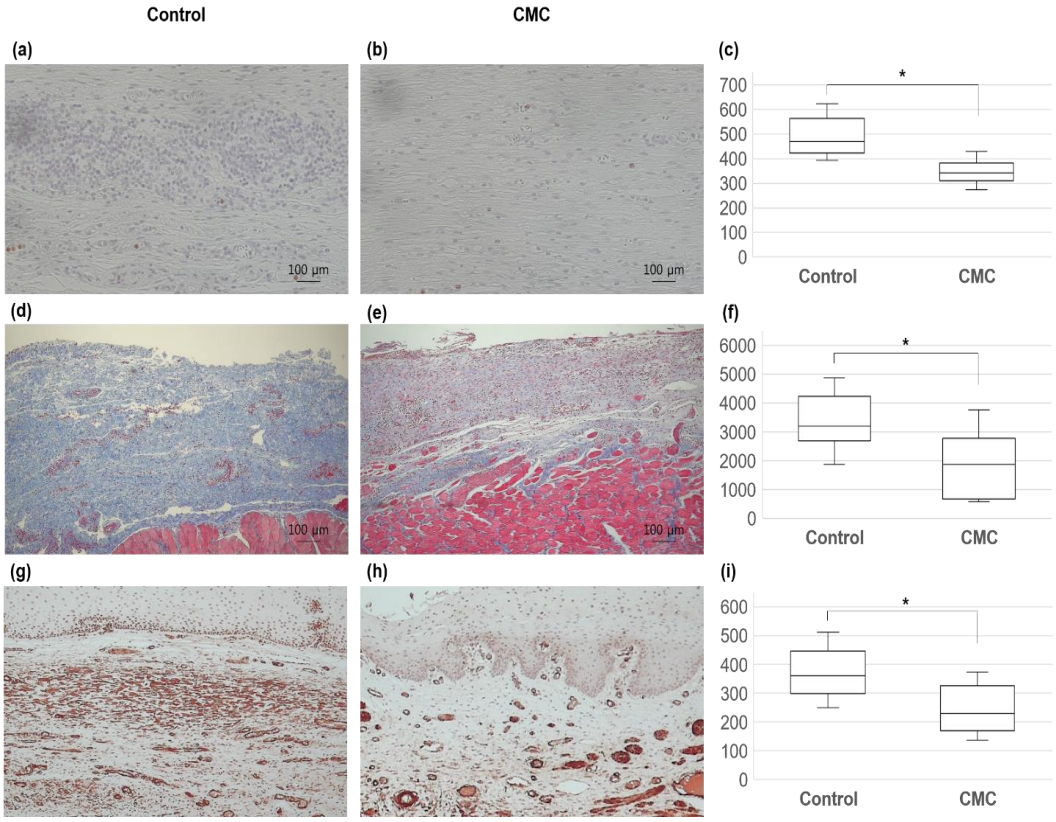

Figure 4. Levels of inflammation and fibrosis in the regenerated esophageal mucosa. (a, b) Massive leukocyte infiltration was observed from the surface to the intermediate layer in the control group compared to the carboxymethyl cellulose (CMC) group (anti-myeloperoxidase antibody, x 200). (c) Inflammatory cell counts per high-power fields between the epithelium and the muscular layer was significantly elevated in control wounds at 4 weeks, compared to CMC wounds. (d, e, f) The thickness of fibrosis (μm) in the submucosal layer was significantly greater in the control group than in the CMC group (Masson trichrome, x 40). (g, h, i) The thickness of α-smooth muscle actin (SMA)-positive myofibroblast bundles (μm) was significantly thinner in the CMC group compared to the control group (α-SMA antibody, x 40). *P value ≤ 0.05.

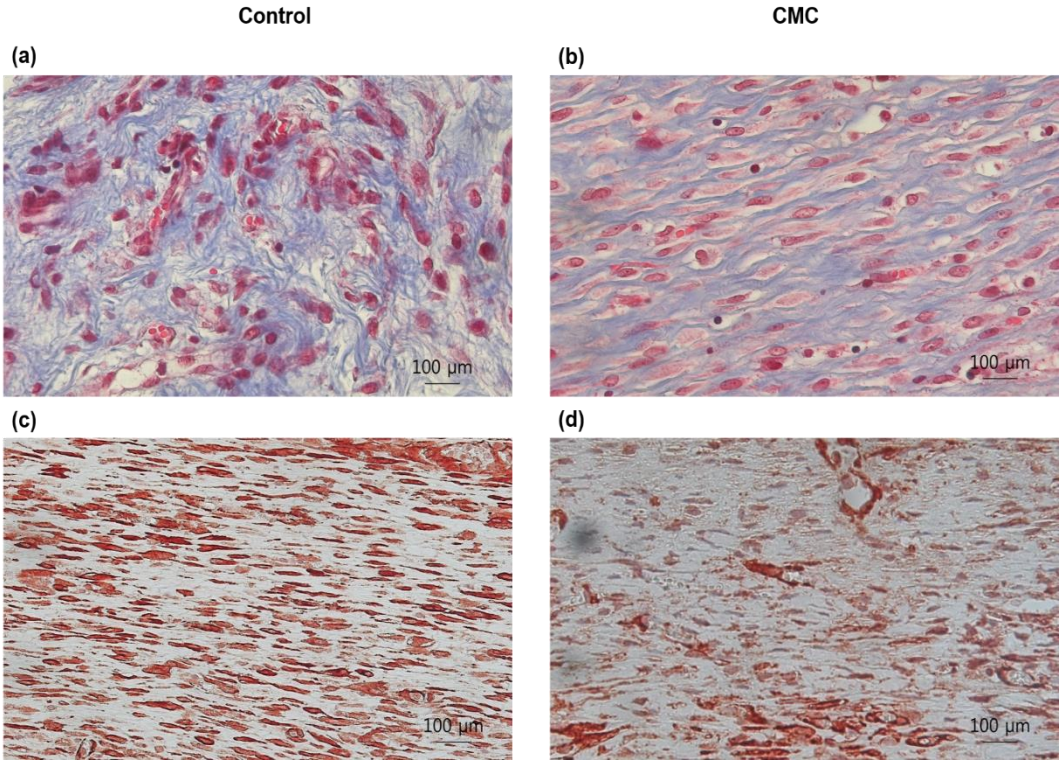

Figure 5. 400x magnification image of the fibrous tissue area. (a, b) The carboxymethyl cellulose (CMC) group exhibited a more organized arrangement of collagen fibers compared to the control group (Masson trichrome, x 400). (c, d) α-smooth muscle actin (SMA) positive myofibroblasts are arranged in a parallel fashion and extend horizontally, whereas in the CMC group, they appear stellate or polyogonal in shape and are arranged haphazardly (α-SMA antibody, x 400).

4. DISCUSSION

Esophageal stricture remains a significant complication following near circumferential ESD, leading to dysphagia and impaired quality of life for patients. Our study investigated the efficacy of CMC sheets in preventing esophageal strictures in a porcine model post-ESD. The findings suggest that CMC sheets may offer a beneficial approach to mitigating the complications associated with near circumferential ESD.

To date, the most commonly recommended and implemented methods for preventing esophageal stricture after ESD are local steroid injections or oral steroid administration in real world. However, the incidence of stricture after local steroid injections varies across

studies, with non-circumferential ESD showing rates between 10 and 56%, [19-25] while total circumferential ESD reports rates of 80% to 100%. [21-23, 25] Notably, the benefits are limited in patients with more than 75% circumference involvement. [26-28] In addition, this injection method is invasive and requires precise skill, with complications such as perforation [29] or injection into the muscle layer potentially leading to necrosis or abscess formation. [30]

Oral steroid administration has been reported to result in stricture rates ranging from 5% to 82%. [13, 31-34] While it does not require special techniques, there are no established guidelines regarding dosage and duration, and its effectiveness is also limited after extensive resections. Additionally, there are concerns about side effects related to systemic absorption of steroids, including hyperglycemia, infections such as pneumonia, and steroid-related myopathy. [32, 35] Moreover, while these local or systemic steroid preparations have an inhibitory effect on scar formation, they also slow down wound healing. This effect is particularly noted to be less effective in long segment defect wounds. [36, 37]

Another approach to preventing esophageal stricture after ESD, aside from steroids, includes stent insertion and cell transplantation-based tissue engineering. Limited studies have reported that prophylactic stenting could reduce the stricture rate. [38] However, stent insertion is associated with common complications, such as stent displacement or migration, and can cause discomfort and pain for the patient. [38] Most importantly, there is the risk of stricture recurrence after stent removal. Cell-based tissue engineering methods require complex techniques, such as cell transplantation and the use of biomaterials. [39] The clinical evidence for this method is still insufficient, and its efficacy needs to be confirmed in comparative studies. [39] Therefore, current methods for preventing stricture are not fully effective, carry various concerns regarding side effects, and none have been proposed as a standard preventive approach.

Our study aimed to investigate the effects of CMC sheets on scar formation from extensive esophageal ulcers after ESD in porcine models. We demonstrate that CMC sheets aid not only in histological healing but also in the recovery of physiological function following near circumferential esophageal ESD.

The CMC sheet is made from biodegradable materials that naturally break down or can be removed from the wound site once healing is complete. The primary ingredient used in the CMC sheets is oxidized regenerated cellulose, a plant-derived, biocompatible, and biodegradable polymer. [40] In this case, CMC, a water-soluble and absorbent derivative of cellulose, is used to enhance its absorbency in the body. [41] Once placed in the body, oxidized regenerated cellulose absorbs blood and moisture from the surgical site and the surrounding area, forming an immediate, colorless, and transparent high-viscosity protective film. [41] This film remains in the tissues for up to two weeks, facilitating

mucosal regeneration. [41] During this time, it helps prevent adhesion between damaged tissues and safely degrades within the body into carbon dioxide and water. [41] It is known for assisting the healing process without causing additional damage to the wound area. [42]

Examining the recovery process after esophageal mucosal injury, an inflammatory response occurs within the first 1-3 days, during which leukocytes accumulate and inflammatory cytokines are secreted. [43] Following this, for more than two weeks, fibroblasts and epithelial cells proliferate actively, beginning to cover the damaged area. [43] Over the subsequent weeks to months, new tissue forms, and connective tissue, such as collagen, accumulates, leading to fibrosis. [43] If the complete regeneration of the epithelial cells in the esophageal mucosa is hindered during this process, scar tissue can develop, contributing to the narrowing of the esophageal lumen. Furthermore, excessive accumulation of extracellular matrix components, such as collagen, due to fibroblast activation can decrease tissue elasticity and impair the motility and flexibility of the esophagus. [44, 45]

In our study, histopathological analysis of the tissue obtained at four weeks indicated a significantly lower stricture ratio and a significantly higher re-epithelization ratio in the CMC group. For the assessment of inflammation and fibrosis, inflammatory cell counts were significantly lower at the wound site and fibrosis thickness was significantly reduced in the CMC group. Additionally, the fibrous tissue area of the CMC group exhibited a more organized arrangement of collagen fibers compared to the control group (**Figure 5 A, B**). The abundance of α-SMA-positive myofibroblast, which play a crucial role in wound contraction, was also significantly lower in the CMC group. Previous research has reported that the appearance and proliferation of α-SMA-positive myofibroblast, showing regular horizontal arrangement, seem to be related to stricture formation. [45] Our study showed similar findings; the arrangement of α-SMA-positive myofibroblasts was parallel in the control group, whereas they were arranged haphazardly in the CMC group (**Figure 5 C, D**).

The impact of CMC sheets on preventing stricture is significant. Initially, after application, CMC sheets can absorb and retain moisture, creating a humid environment that promotes faster migration of epithelial cells and aids in angiogenesis, allowing for quicker re-epithelialization. Additionally, CMC sheets act as scaffolds, helping to maintain tissue in a fixed position at the wound site, thereby minimizing wound contraction. They also serve as a barrier, effectively absorbing exudates due to their adhesive properties, which reduces the risk of infection. This helps inhibit the activity of inflammatory cytokines and mitigates excessive immune responses, thereby reducing fibroblast migration and the side effects associated with chronic inflammation, such as fibrosis. Moreover, the adherence of the

sheets to the wound can promote coagulation, assisting in hemostasis.

Compared to steroids, which inhibit the infiltration of inflammatory cells and collagen deposition while also hindering re-epithelialization. [46, 47] CMC sheets primarily act toward promoting wound healing, which may be more advantageous.

A notable feature of this study is the comparison of the esophageal distensibility using EndoFLIP.® The EndoFLIP® device uses impedance planimetry to measure the serial cross-sectional areas, pressure, and tension-strain relationship within the esophageal lumen. [48] This allows for the assessment of luminal distensibility and the contractile function of the esophageal wall. [49, 50] By utilizing EndoFLIP®, it is possible to measure the real-time peristaltic movement of the esophagus, providing an evaluation of the extent to which contractile function is maintained in the healing area of the mucosal defect. The DI value serves as a useful indicator for evaluating the physiological function of the esophagus. Our results showed the CMC group had a significantly higher DI value compared to the control group at week 4. This indicates that the attachment of the CMC sheet effectively contributed not only to histological markers but also to maintaining the actual motility function of the esophagus. In wound healing process, fibrosis due to the excessive production of extracellular matrix components, such as collagen, is associated with esophageal wall stiffness. The initial anti-inflammatory effect of the CMC sheet may gradually prevent the activation of fibroblasts, leading to reduced fibrosis and contributing to the maintenance of esophageal tissue elasticity. On the other hand, the minimal diameter of the esophagus was significantly larger in the CMC group compared to the control group at week 2. Luminal narrowing is mainly associated with incomplete epithelialization of the mucosa. During the re-epithelialization process, CMC sheet aids in the migration and proliferation of epithelial cells by providing a moist environment. However, this beneficial function of CMC sheet may gradually plateau after week 2, leading to a reduced role in facilitating mucosal epithelialization. Future studies will be needed to determine the appropriate replacement interval for CMC sheets.

This study has several limitations. First, although the results showed significant differences between the two groups, the sample size was small. Second, the use of a porcine model, while valuable, may not fully replicate human esophageal physiology. Third, the follow-up period of four weeks may not capture the long-term efficacy of CMC sheets in preventing strictures. Future studies should consider longer follow-up durations and larger sample sizes to validate these findings and assess their clinical applicability in human patients.

5. CONCLUSION

Our study supports the hypothesis that CMC sheets can significantly reduce the incidence of esophageal strictures following near circumferential ESD. By promoting re-epithelialization and reducing inflammation and fibrosis, CMC sheets represent a promising strategy for improving postoperative outcomes in patients undergoing ESD. Further research is warranted to explore their potential application in clinical settings.

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Abstract in Korean

카복시메틸 셀룰로오스 시트가 광범위한 내시경 점막하 절제술 후 식도 협착 예방에 미치는 효과: 돼지 모델 연구

서론: 식도 협착은 넓은 범위의 내시경 점막하 절제술(ESD) 후 발생 가능한 주요한 합병증이다. 임상 지침은 식도 ESD 후 식도 내강 둘레의 75% 이상에서 점막 결손이 발생한 환자에서 협착 방지를 위한 치료를 권고하고 있지만 현재까지 안전하고 효과적인 예방법은 정립되지 않았다. 카복시메틸 셀룰로오스 (Carboxymethyl Cellulose) 시트는 식물 셀룰로오스에서 유래한 생체분해성 물질로 혈액이나 수분과 접촉하면 젤을 형성하며 조직에 부착되어, 염증 반응을 줄이고, 상처 치유에 도움이 되는 환경을 유지하도록 한다. 본 연구는 카복시메틸 셀룰로오스 시트가 돼지 모델에서 광범위한 ESD 후 식도 협착 예방에 미치는 효과를 연구하고자 하였다.
방법: 16마리의 돼지에게 5cm 길이의 식도 내강 둘레의 75% 이상을 차지하는 ESD를 시행하고, 카복시메틸 셀룰로오스군 또는 대조군으로 무작위 배정하였다. 카복시메틸 셀룰로오스군은 ESD 직후 점막 결손부를 카복시메틸 셀룰로오스 시트로 완전히 덮도록 부착하였고, 대조군은 ESD만 시행하였다. ESD 후 1, 2, 4주차에 내시경 및 기능성 관류 영상 프로브(EndoFLIP®) 평가를 하였고, 4주차에 돼지를 안락사 시킨 후, 거시적 및 조직학적 평가를 수행하였다.
결과: 카복시메틸 셀룰로오스군은 대조군에 비해 협착률이 유의미하게 낮았다(53.5% vs 69.2%, p<0.001). 4주차 EndoFLIP® 평가에서 카복시메틸 셀룰로오스군은 대조군에 비해 유의미하게 높은 확장 지수(distensibility index)를 보였다(0.30 mm^2 /mmHg vs 0.18 mm^2 /mmHg, p=0.005). 조직학적 분석 결과, 카복시메틸 셀룰로오스군은 유의미하게 높은 재상피화 비율(0.54 vs 0.34, p=0.015)과 낮은 염증 세포 수(346.2 vs 486.0, p=0.001), 낮은 섬유화 두께(1841.8 μm vs 3392.3 μm, p=0.015)를 나타냈다.
결론: 카복시메틸 셀룰로오스 시트는 ESD 후 상처 회복 과정에서 식도의 생리적 기능을 보존하는 데 유리한 효과를 보여주었다. 카복시메틸 셀룰로오스 시트의 사용은 광범위한 ESD 후 식도 협착을 예방하는 효과적인 전략이 될 수 있을 것이다.

핵심되는 말: 식도 협착, 내시경 점막 하 절제술, 카복시메틸 셀룰로오스, 예방