

## EXPERIMENTAL STUDY

# The effect of hyaluronic acid carboxymethyl cellulosis on the healing of colonic anastomosis in rats

Gokhan Adas<sup>1</sup>, Oguzhan Karatepe<sup>1</sup>, Soykan Arýkan<sup>2</sup>, Muharrem Battal<sup>1</sup>, Ozgur Kemik<sup>2</sup>, Merih Altiok<sup>1</sup>, Gulcin Kamali<sup>3</sup>, Servet Karahan<sup>1</sup>

Istanbul Okmeydani Training Hospital 2nd General Surgery Department, Istanbul, Turkey. [drkaratepe@yahoo.com](mailto:drkaratepe@yahoo.com)

**Abstract:** *Background:* This study demonstrates the effect of hyaluronic acid-carboxymethylcellulose on the healing of colonic anastomosis.

*Method:* 30 female Wistar-Albino rats were divided into three groups; control group 1 (n=10), treated with hyaluronic acid-carboxymethylcellulose, group 2 (n=8) treated with HBO and group 3 with hyaluronic acid-carboxymethylcellulose and HBO.

*Result:* Bursting pressure and rupture strength were significantly higher in the group 3 compared to other two groups ( $p < 0.05$ ). The severity of necrosis, granulation, neovascularisation and epithelization among groups did not show any significant difference ( $p > 0.05$ ). But the evaluation of inflammation showed a statistical significance ( $p < 0.001$ ) such as as granulation ( $p < 0.05$ ).

*Conclusion:* This study did not detect the negative affect of seprafilm on wound healing. Combined treatment with seprafilm and HBO has a favorable therapeutic effect on the healing of ischemic colonic anastomosis (Tab. 4, Fig. 3, Ref. 15). Full Text (Free, PDF) [www.bmj.sk](http://www.bmj.sk).

**Key words:** hyaluronic acid carboxymethyl cellulosis, wound healing, colon anastomosis.

The anastomosis leakage in gastrointestinal system generally occurs in the colon and the incidence of leakage increases in distal parts (2, 5). The healing process is very important when the anastomosis leakage occurs. This process involves complex biochemical and cellular steps, with the collagen synthesis being of fundamental importance. The basic mechanisms involved in the healing process are: aggression, inflammation, collagen metabolism, epithelialization, and wound contraction. After aggression, which in this study consisted of colonic anastomosis, the first stage involves mechanisms of vasoconstriction and coagulation, with the formation of thrombus and local platelet activation. The activated platelets release cytokines such as platelet-derived growth factor (PDGF), corresponding to the primordial stimulus for cell chemotaxis, transforming growth factor beta (TGF- $\beta$ ), platelet activating factor (PAF), fibronectin, and serotonin. PAF stimulates the arrival of fibroblasts as well as polymorphonuclear cells, mast cells and macrophages, which complement the inflammatory reaction. This equilibrated inflammation is fundamental for the healing. The mediators dilate local capillaries, increase vascular permeability, white cell diapedesis and

macrophage and neutrophil taxis into the wound. Then the macrophages promote phagocytosis of foreign matter, bacteria and clots, in addition to release of their own cytokines. They also play a fundamental role in fibroblast activation and stimulation for collagen synthesis. 39HA-CMC (Seprafilm) is composed of sodium hyaluronate and carboxycellulose and used to prevent and decrease the adhesivity after operations. Although hyaluronic acid-carboxymethylcellulose (HA-CMC) membrane has the advantage of preventing intraabdominal adhesions, it has a theoretical risk of negative effects on the healing of intestinal suture lines by forming a barrier between the suture lines and neighboring serosal surfaces.

In this study, the affect of Seprafilm mesh on wound healing was studied on an experimental animal model. Our aim was to detect the useful and useless effect of mesh therefore the early phase of wound healing was studied.

## Materials and methods

This study was performed on 30 Wistar Albino rats of about 200–250 g. weight at the animal laboratory of Submarine and Hyperbaric Section of Istanbul University Medical Faculty. The study was approved by the local ethic committee. Totally 30 rats were utilized in each group containing 10 rats. These were held at room temperature at metallic cage and fed by tap water at 12th hour and by provender at 24th hour postoperatively.

Only colon anastomosis was applied to first group and accepted as control group. In the second group, colon anastomosis

<sup>1</sup>Istanbul Okmeydani Training Hospital 2nd General Surgery Department, <sup>2</sup>Haseki Teaching Hospital General Surgery Department, and <sup>3</sup>Istanbul Okmeydani Training Hospital Pathology Department, Istanbul, Turkey

**Address for correspondence:** Oguzhan Karatepe, Istanbul Okmeydani Training Hospital 2. General Surgery Department, 34340, Sisli, Istanbul, Turkey.

Phone: +90.212.2217777, Fax: +90.2163612140

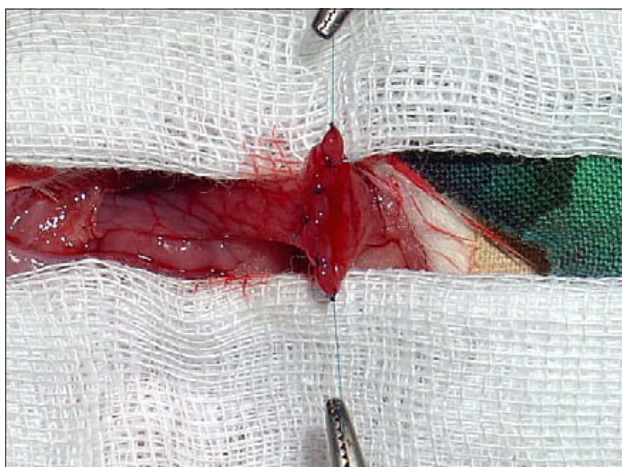


Fig. 1. Practice of colon anastomosis.

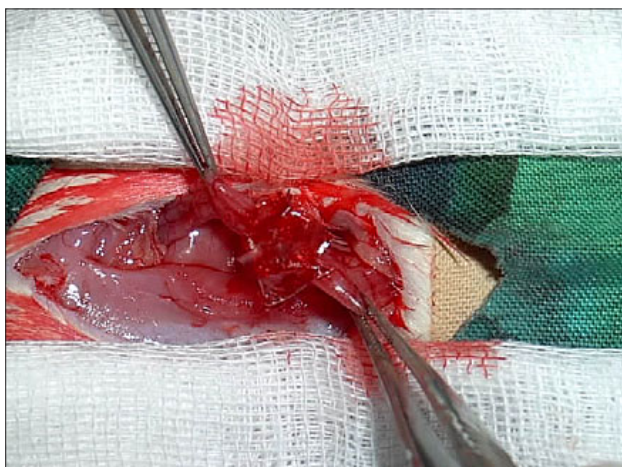


Fig. 2. Placed Sefrafilim around the colon anastomosis (Group 2).

and seprafilim application around the anostomosis were placed, and in third group, after anostomosis, seprafilim was placed under the peritoneum.

All rats were put to death 72 hours post-operatively using the ketamine anesthesia. Mechanical resistance of anastomosis was measured by bursting pressure and early phase of wound healing was evaluated histopathologically. The results were evaluated statistically and  $p < 0.05$  was accepted as significant.

### Surgical procedures

Operations were performed under sterile conditions. After shaving the abdominal hair, skin cleaning was made by povidine iodine (10 %). After one night fasting, the animals were anaesthetized by an intramuscular injection of ketamine hydrochloride (50–100 mg per kg of body weight). Median laporatomic incision, 4 cm long, was performed and after a bleeding control colon was cut 3 cm. proximally of the peritoneal reflexion in the



Fig. 3. Severe omental adhesions around the anastomosis (Group 2).

first group. After the stool content had been milked out, a standardized end to end anastomosis was made with eight interrupted, inverting sutures of 6/0 polypropylene. In the second group, anastomosis sites were covered loosely with seprafilim of 1x3 cm in size. (Sefrafilim II- Adhesion Barrier Genzyme Corporation U.S.A.). In the third group, after the anastomosis, Sefrafilim was laid under the peritoneum in size of 4x2 cm. After the bleeding control had been done, 2cc 0.9 % NaCl was injected intraperitoneally and abdomen was closed with 2/0 silk continual suture.

Due to the disappearance of the Sefrafilim at 72 hours post-operatively, all the rats were put to death under ketamine anaesthesia. The abdominal incision was reopened and anastomotic sutures line was found. The anastomosis segment was cut out, preserving the adhesions which were 2 cm proximal and 2 cm distal to the anastomosis line. The bursting pressure of the anastomosis segment was measured with a mercury monometer and a constant flow pump. Briefly, 16 gauge silastic catheter was inserted via colotomy in the proximal colon and ligated with 2/0 silk tie. The rectum distal to the anastomosis was ligated with 2/0 silk and was continuously infused through the catheter via a tube pump at a rate of 4 ml/minute. Bursting pressure was measured in a water filled container and recorded as the peak pressure attained before the rupture of the anastomosis, which was observed with the bubbling of the air and abrupt drop in pressure. After bursting, the colon was excised, cleared of surrounding mesentery and fat, and washed with saline. The anastomosis, together with 1 cm of adjacent proximal and distal colon, was excised. The segment of bowel was opened longitudinally, fixed in 10 percent formalin approximately 24 hours then embedded in parafin. Transverse sections of the embedded tissue 3  $\mu$ m thick were stained with haematoxylin and eosin, and histological evaluation was carried out without knowing the group type. Necrosis, ephitelization, inflammatory process, granulation, collagen deposition and neovascularization at the anastomotic site was examined and histological scored as follows: 0: none, 1: slightly, 2: moderate, 3: densely (Figs 1, 2 3).

**Tab. 1. The bursting pressures of each group (mmHg).**

n=10	Bursting pressures		
	Group 1	Group 2	Group 3
1	190	200	180
2	195	210	185
3	240	200	195
4	180	225	195
5	195	205	185
6	190	160	185
7	230	205	185
8	220	200	190
9	210	210	195
10	205	160	190
Mean	205.5	197.5	188.5
Median	200	202.5	187.5

## Results

Totally, results of 30 rats were evaluated. Regarding to Mann-Whitney U test, results were evaluated among groups and there was no difference between the group I and group II ( $p>0.05$ ). In contrast, the differences were significant between the group I and group III and also between the group II and group III ( $p<0.05$ ) (Tabs 1, 2, 3, 4).

## Discussion

Colon anastomosis shows great differences in morbidity and mortality in the world, so many experiments with different kinds of materials were used to prevent leakage in the colon anastomosis (1–4). The leakage from anastomosis in the gastrointestinal system generally occurs in the colon (2, 5). Loose collateral branches of arteries and rich bacterial flora at the distal portion of the colon also facilitate the leakage after surgical operations (6). Additional problems increase the risk of complications in colon surgery (1–4). Developments on preoperative and postoperative care and operation technique decreased the morbidity about 9 %, and mortality about 3–5 % (9).

Regarding to literature, occurrence of leakage from anastomosis ranges between 3 to 8 % percent. The main principles of anastomosis depend on the effective gut clearance, efficient blood supply to edges, low resistance in the ends of lumen, avoidance of infection, surgical techniques that ensure to convert the ends of lumen inside and coverage with omentum (11). The age of patient, malnutrition, obesity, uremia and steroids also affect the anastomosis site (5, 11).

HA-CMC decreases the occurrence of strictures but it is not well known that decreasing the stricture occurrence is useful in preventing the leakage and it is still unknown that it may cause more clinical complications (12). Because, these bio absorbent materials function as a barrier between the traumatic mesothelial tissues, it is accepted that they also isolate sutures from serosal surfaces and decrease wound healing while decreasing the

**Tab. 2. The data of bursting pressures. Histological evaluation.**

	Group 1	Group 2	Group 3
n	10	10	10
Mean	205.50	197.50	188.50
Median	200.00	202.50	187.50
Standard deviation	19.35	21.11	5.29
Range	60.00	65.00	15.00

stricture occurrences. Meanwhile, these materials may also cause foreign material reactions and inflammation, so they affect wound healing negatively.

Oosterom examined the affect of HA-CMC on healing the intraabdominal and extraabdominal small intestine anastomosis in rats (13). And HA-CMC was shown to have no negative affect on the healing of intraabdominal and extraabdominal small intestine anastomosis.

In the Bowers study, a formation of abscess around the small intestinal HA-CMC applied anastomosis was clearly high in rats given a radioactive substance (14).

The usage of HA-CMC on the intestinal anastomosis in rabbits was shown to have no negative affect in the study of Medina et.al (15).

In the study of Reijen et al, it was proposed that Seprafilm had no affect on healing of colon anastomosis whether bacterial peritonitis was found or not (16). On the other hand, it is known that ischemia and mechanical trauma at the edges of intestine negatively affect wound healing (17). In the study of Högström et al, strained edges of intestine were shown to cause cellular inflammation and decrease wound healing (18).

Seprafilm is a transparent material and it loses its film property on tissues in a short time. It is laid between the internal organs and abdominal wall at the end of laparoscopic operations (19).

In this study, regarding the intestinal sutures rupture pressure, there were significant differences among the three groups ( $p<0.05$ ). When compared to the control group (group I) and the group covered with Seprafilm (group II), rupture pressure was markedly high in favor of the group II ( $p<0.05$ ). This study showed that the formation of strictures was not decreased but increased. In the histopathological investigation, the severity of inflammation was high in the group II ( $p<0.05$ ). These findings were in accord with increased stricture formation. The rupture pressures were low in the group of containing Seprafilm under the peritoneum (group III) compared to other groups. In the second group, intraabdominal strictures were very high and common and the ruptures of the anastomosis had not occurred at the site of sutures, some of them occurred at a distance of 1–2 cm.

In the histopathological examination, the severity of necrosis, granulation, neovascularisation and epithelization among groups did not show big differences ( $p>0.05$ ). In the evaluation of inflammation, the results showed highly significant differences ( $p<0.001$ ), but there were not big differences between the group I and group III ( $p>0.05$ ). In the evaluation of granulation forma-

**Tab. 3. The median value of the histopathological scores. Statistical evaluations were performed by GraphPad Prisma V.3 programme. When evaluating quality values, q square test was used.  $p < 0.05$  was accepted as significant.**

Group	Necrosis	Epitelization	Inflammation	Granulation	Collagen synthesis	Neovascularization
1	0.9	0.2	1.8	1.7	0.3	1.8
2	1.8	0.4	2.8	2.2	1.7	2.1
3	1.1	0.3	2.0	1.3	1.4	1.7

**Tab. 4. The statistical evaluation of the histopathological data. The severity of necrosis, granulation, neovascularisation and epitelization among groups did not show big difference ( $p > 0.05$ ). But in the evaluation of inflammation, differences were significant ( $p < 0.001$ ) such as granulation ( $p < 0.05$ ).**

	Score	Group 1	Group 2	Group 3	
Necrosis	0	3 (30%)	1 (10%)	1 (10%)	chi <sup>2</sup> :10.31 p>0.05
	1	5 (50%)	2 (20%)	7 (70%)	
	2	2 (20%)	5 (50%)	2 (20%)	
	3	0 (0%)	2 (20%)	0 (0%)	
Epitelization	0	7 (70%)	7 (70%)	7 (70%)	chi <sup>2</sup> :2.25 p>0.05
	1	3 (30%)	2 (20%)	3 (30%)	
	2	0 (0%)	1 (10%)	0 (0%)	
Inflammation	1	2 (20%)	0 (0%)	1 (10%)	chi <sup>2</sup> :18.66 p<0.001
	2	8 (80%)	2 (20%)	8 (80%)	
	3	0 (0%)	8 (80%)	1 (10%)	
Granulation	0	0 (0%)	0 (0%)	1 (10%)	chi <sup>2</sup> :12.6 p<0.05
	1	3 (30%)	2 (20%)	5 (50%)	
	2	7 (70%)	4 (40%)	4 (40%)	
	3	0 (0%)	4 (40%)	0 (0%)	
Collagen synthesis	0	7 (70%)	0 (0%)	0 (0%)	chi <sup>2</sup> :21.74 p<0.0001
	1	3 (30%)	4 (40%)	6 (60%)	
	2	0 (0%)	5 (50%)	4 (40%)	
	3	0 (0%)	1 (10%)	0 (0%)	
Neovascularization	1	3 (30%)	2 (20%)	4 (40%)	chi <sup>2</sup> :2.39 p>0.05
	2	6 (60%)	5 (50%)	5 (50%)	
	3	1 (10%)	3 (30%)	1 (10%)	

tion, group II showed significant results ( $p < 0.05$ ) but there were not big differences between the group I and group III ( $p < 0.05$ ). In the evaluation of collagen accumulation, group II and group III showed big differences from the group I ( $p < 0.0001$ ) but there were not big differences between the group II and group III ( $p > 0.05$ ).

In literature, the positive effect of Seprafilm on wound healing and decrease of stricture formation is indicated. In this study, especially in the group with Seprafilm around the anastomosis, regions of high degree accumulation of inflammation, granulation and collagen were shown. High rupture pressures were also found in this group. The reason might be due to occurrence of high degree strictures in the anastomosis region by serosal surfaces and neighbor organs. Seprafilm detached the anastomosis region from environment and might cause a decrease in healing and an increase in inflammation and granulation. In the evaluation of the control group and the group containing Seprafilm under the peritoneum (group III), there were not big differences in inflammation and granulation at the region of anastomosis. As in the previous studies, Seprafilm was shown harmless (13, 17).

In summary, this study did not detect the negative affect of Seprafilm on wound healing. In contrast, it was found useful. It should be mentioned that Seprafilm did not decrease the strictures in the abdominal cavity. For this reason, extensive studies including more parameters are needed. In this study, the early phase of wound healing was evaluated. For this purposes, studies on both early and late phase of wound healing may be useful. In the evaluation of the effect of Seprafilm on colon anastomosis, further clinical and experimental studies are needed.

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