



A Novel Method of Comparing Anti-Adhesive Agents Used to Prevent Postoperative Peritoneal Adhesions

Post-Operatif Peritoneal Adezyonları Önlemek için Kullanılan Anti-Adeziv Ajanların Karşılaştırılmasında Yeni Bir Yöntem

Engin Hatipoğlu¹, Süleyman Demiryas¹, Akif Enes Arıkan¹, Anıl Orhan¹, Zafer Küçükodacı², Sabri Ergüney¹, Tarık Akçal¹

¹Istanbul University Cerrahpaşa Faculty of Medicine, Department of General Surgery, İstanbul, Turkey

²University of Health Sciences, Haydarpaşa Numune Training and Research Hospital, Clinic of Pathology, İstanbul, Turkey

ABSTRACT

Aim: In this study, our aim was to utilize a novel and objective method of adhesion evaluation and compare the effectiveness of anti-adhesive properties of commonly used agents, such as sodium hyaluronate and carboxymethylcellulose membrane (HA-CMC), polyethylene glycol lysine (PEG-L), and sodium hyaluronic acid (HA).

Method: Cecal abrasion was performed on 64 female Wistar albino rats (250-300 g) which were randomized into two equivalent groups, one group was sacrificed on postoperative day 5 (group 1), and the other on postoperative day 14 (group 2). Each group was subcategorized to four groups according to applied anti-adhesive material: PEG-L, sodium HA-CMC, HA, and control group. Adhesions were evaluated with conventional methods and the novel "Zone Calculation" method.

Results: In group 1, no significant differences were found with any of the evaluation tools. However, in the PEG-L subgroup of the group 2, adhesion zone score was higher with the zone calculation method. The same subgroup was found to have a higher inflammation score on histopathological evaluation; however, other results were similar.

Conclusion: Although histopathological results and macroscopic evaluations were similar in all groups, the zone calculation method indicated greater adhesion in the PEG-L subgroup. Conventional evaluation methods of adhesion are considered to be semi-quantitative, whereas the zone calculation method does not allow subjectivity. We believe this method can replace conventional methods and can be used in studies evaluating anti-adhesive agents/materials.

Keywords: Sepracoat, seprafilm, SprayShield, preventing adhesions, animal experiment

ÖZ

Amaç: Bu çalışmada amacımız yeni bir objektif adezyon değerlendirme yönteminden yararlanmak ve sodyum hiyalüronat ve karboksimetilselülöz membran (HA-CMC), polietilen glikol lizin (PEG-L) ve sodyum hiyalüronik asit (HA) gibi sıklıkla kullanılan anti-adeziv ajanların etkinliğini karşılaştırmaktır.

Yöntem: Çekum abrazyonu, bir kısmında post-op 5. günde (grup 1) ve diğerlerinde post-op 14. günde (grup 2) oluşturulmak üzere iki eşdeğer gruba randomize edilen 64 dişi Wistar albino sıçanında (250-300 g) uygulandı. Her bir grup, uygulanan anti-adeziv ajana göre; PEG-L, HA-CMC, HA ve kontrol grubu olmak üzere dört alt gruba ayrıldı. Adezyonlar, geleneksel yöntemlerle ve yeni bir yöntem olan "Bölge Hesaplama" yöntemi ile değerlendirildi.

Bulgular: Birinci grupta herhangi bir değerlendirme aracı ile anlamlı fark bulunamadı. Bununla birlikte, ikinci grubun PEG-L alt grubunda, bölge hesaplama yöntemi ile yapışma bölgesi skoru daha yüksekti. Aynı alt grubun histopatolojik değerlendirmesinde daha yüksek enflamasyon skoru bulundu; ancak, diğer sonuçlar benzerdi.

Sonuç: Histopatolojik sonuçlar ve makroskopik değerlendirmeler tüm gruplarda benzer olmakla birlikte, bölge hesaplama yöntemi ile PEG-L alt grubunda adezyon artışı gösterildi. Yapışma konvansiyonel değerlendirme yöntemleri yarı kantitatif olarak kabul edilirken, bölge hesaplama yöntemi subjektif değerlendirmelere izin vermemektedir. Bu yöntemin geleneksel yöntemlerin yerini alabileceğine ve anti-adeziv ajanları/materyalleri değerlendiren çalışmalarda kullanılabileceğine inanıyoruz.

Anahtar Kelimeler: Sepracoat, seprafilm, SprayShield, yapışıklık önlenmesi, hayvan deneyi



Address for Correspondence/Yazışma Adresi: Engin Hatipoğlu MD

İstanbul University Cerrahpaşa Faculty of Medicine, Department of General Surgery, İstanbul, Turkey

Phone: +90 505 539 18 77 E-mail: enginhatipoglu@yahoo.com ORCID ID: orcid.org/0000-0002-6824-455X

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Introduction

Post-operative peritoneal adhesions (PAPA) are very frequent (95%) after abdominal surgery.¹ During the normal healing process of tissue, fibrin is degraded enzymatically and mesothelial injury is healed in seven days.¹ If this fibrin is not degraded, it will cause the development of adhesions between intra-abdominal surfaces and will lead to formation of fibrous adhesions and bands. Bowel obstruction, abdominal pain, infertility due to adhesions after surgery can lead to the requirement of further surgery. These adhesions are responsible for 75% of all bowel obstructions in western countries.² Intra-abdominal adhesions mostly occur in the early post-operative period; thus studies are mostly focused on perioperative precautions. In current literature, biologically degradable physical barriers have acceptable results in preventing the formation of fibrous matrix and intra-abdominal adhesions.² However, adhesions remain as a problem in 50% of patients.³

To solve this problem, a wide range of materials, agents and methods have been utilized. Various non-absorbable (e.g. amniotic membrane) or absorbable at body temperature (e.g. hyaluronic acid and derivatives, oxidized cellulose) mechanical barriers have been tested in addition to more recent biodegradable membranes such as poly(lactide-co-glycolide) (PLGA) membranes.⁴ Agents such as steroids, hyaluronic acid, and dextrin have also been utilized.⁵ In other studies, liquids such as olive oil, soy oil, glycerol, and honey are shown to have varying degrees of effect.⁶ Additionally, the effects of ringer lactate and serum physiologique (saline) have been investigated.⁷

With the development of anti-adhesive agents, decrease in the frequency of adhesion has been achieved to some extent. However, determination of the most effective agent in the prevention of adhesion remains a controversial matter. The results of numerous anti-adhesive materials in the literature are conflicting, almost every material has been “shown” to be better than the rest.^{8,9,10} However, in most of these studies, success was determined by macroscopic evaluation and/or the use of semi-quantitative histopathological classification. While these materials and other precautions may decrease adhesion development, there is a need for better objective evaluation of adhesions and long-term studies.

In this study, our aim was to evaluate and compare the results of various methods used for the prevention of intra-abdominal adhesion, such as the commonly used sodium hyaluronate and carboxymethylcellulose membrane (HA-CMC), polyethylene glycol lysine (PEG-L), and sodium hyaluronic acid (HA). These methods were compared with the “Zone Calculation” method, an objective novel parameter, beside conventional methods.

Materials and Methods

Sixty-four female Wistar albino rats (250-300 g) were obtained from İstanbul University, Experimental Medicine Research Institute, Animal Experiments Laboratory. An ethics committee approval was taken from İstanbul University Local Ethics Committee (approval number: 2011/54) for the study.

They were randomized into two equivalent groups, one group was sacrificed at the 5th post-op day, and the other at the 14th post-op day. Each group was subcategorized to four groups according to applied anti-adhesive material; PEG-L, sodium HA-CMC, sodium HA, and sham (control group) (Figures 1, 2, 3, 4).

Animals were fed *ad libitum* and kept as 4 per cage in 18-23 °C room temperature, 50-55% humidity, 12h/12h day and night cycle. Oral feeding was ceased before the procedure. Cecal abrasion was performed according to

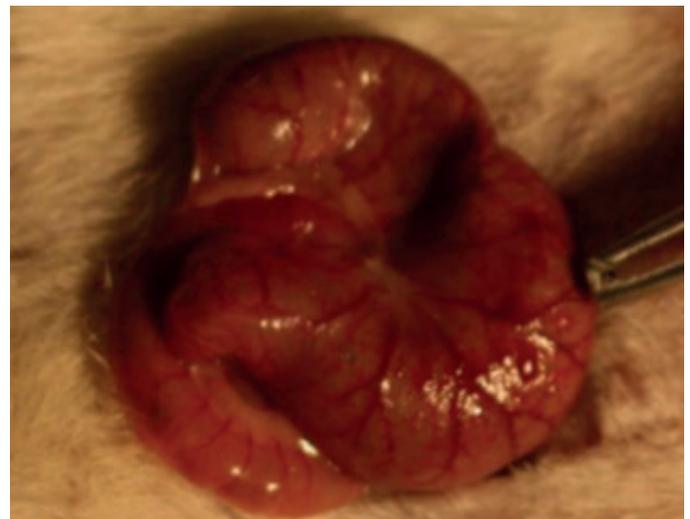


Figure 1. Creating abrasions



Figure 2. Sefrafilim application (hyaluronate and carboxymethylcellulose membrane)

the modified rat cecal and abdominal wall abrasion model approved by Massachusetts General Hospital, Research Animal Care Committee.¹¹ After ten minutes of drying following the application of cecal abrasion and anti-adhesive materials, incision was sutured. Group 1 was sacrificed in post-operative fifth day and group 2 in 14th day. Obtained samples were evaluated by surgeons and pathologists who were blinded to the study protocol.

Histopathological Sampling

After sacrifice, all samples obtained from the abrasion area were buffered in phosphate solution and fixed in 10% buffered formaldehyde solution overnight. Following fixation in paraffin blocks and cutting of sections, samples were dyed with hematoxylin-eosin and evaluated by fibrosis score, inflammation score, vascular proliferation score and the novel “Zone Calculation” method (Figures 5, 6).

Macroscopic and Microscopic Measurements

A blinded surgeon macroscopically determined the degree of adhesion according to the Mazuji classification.¹² Mazuji classification grades adhesion on a scale of 0-4: grade 0: no adhesion, grade 1: small and irregular adhesions, grade 2: mid-dense and easily detachable adhesions, grade 3: dense



Figure 3. Sepracoat application (hyaluronic acid)



Figure 4. SprayShield application (polyethylene glycol lysine)

and hardly detachable adhesions, and grade 4: very dense and hardly detachable homogenous adhesions.

The parameters of histopathological evaluation were fibrosis score, inflammation score, and vascular proliferation score. Fibrosis score is graded from 0 to 3 as follows: 0: no fibrosis, 1: loose and minimal fibrosis, 2: mid-dense fibrosis, and 3: very dense fibrosis. Inflammation score is graded from 0 to 3 as follows: 0: no inflammation, 1: giant cells with sparse lymphocytes and plasma cells, 2: existence of giant cells, plasma cells, eosinophils and neutrophils, 3: existence of numerous inflammatory cells and micro-abscesses. Vascular proliferation score is graded on a 0-3 scale: 0: no vascular proliferation, 1: mild vascular proliferation, 2: moderate vascular proliferation, and 3: dense vascular proliferation.^{13,14}

“Zone Calculation” Method

The distinguishing property of this study is the use of this novel and objective method for evaluation of adhesions. Every hematoxylin-eosin dyed slide of each sample was analyzed with DP2-BSW image analysis software via 20x magnification under Olympus BX53 (Japan) light microscope. A picture of the lesion area was captured from each slide and Zone Calculation was performed. The area for adhesion zone and ratio calculation was standardized as 10 mm².

Statistical Analysis

Kruskal-Wallis test was utilized via NCSS/PASS 2000 (NCSS Statistical Software-Power Analysis and Sample Size, Kaysville, Utah) software.

Results

There were no mortalities and no evidence of obstruction due to adhesion in either group. The comparisons of agents were done separately for each group (group 1: 5th day sacrifice, group 2: 14th day sacrifice).

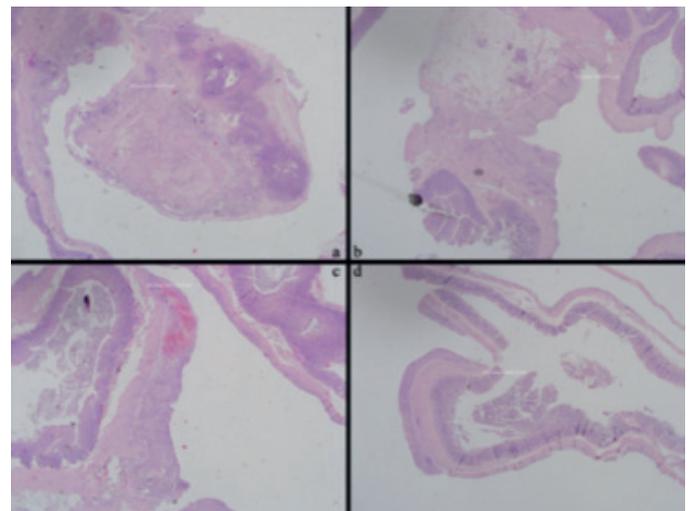


Figure 5. Histological “Zone Calculation” (5th day after the surgery)

Macroscopic and Microscopic Measurements

The results of subjects that were sacrificed on the 5th day and 14th day were compared separately according to the Mazuji classification and we found no significant differences between agents ($p>0.05$). Adhesion grades and detailed results of the Mazuji classification is denoted in Table 1 and 2 (Figure 7).

In terms of histopathological findings, there were no statistical differences for fibrosis score, vascular proliferation score and foreign body reaction between materials in both the 5th and 14th day sacrifice groups ($p>0.05$ for each). In analysis of inflammation score, there were no statistical differences between the agents for those sacrificed on the 5th day. However, in subjects sacrificed on the 14th day, inflammation score was higher in PEG-L applied subjects. Inflammation scores are given in Table 3.

“Zone Calculation” Method

There was no difference between materials for subjects sacrificed on the 5th day ($p>0.05$ for each). However, among those sacrificed in the 14th day, we found adhesion to be higher in the PEG-L group when compared with other materials ($p=0.005$). Detailed measurements are given in Table 4.

Discussion

PAPA is a serious problem in abdominal surgery. After laparotomy, PAPA develops in almost 90% of abdominal surgeries and leads to intestinal obstruction in 3% of those cases.^{15,16} In our study, we found that the evaluation of agents/materials via conventional methods yielded similar results for all agents. The histopathological evaluation showed that inflammation score was higher in the PEG-L group in those sacrificed on the 14th day. However, other histopathological results were similar. Results with the Zone Calculation method were also similar for all agents/materials in those sacrificed on the 5th day. However, with this method, the

PEG-L group was shown to have worse adhesion compared to other materials in the 14th day sacrifice group.

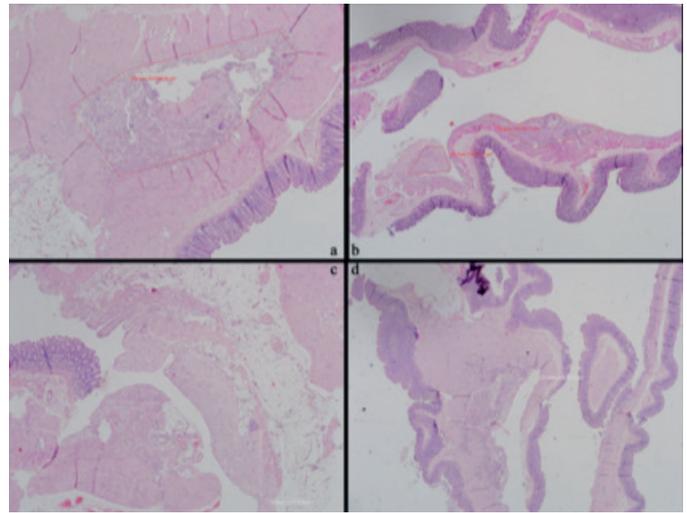


Figure 6. Histological “Zone Calculation” (14th day after the surgery)



Figure 7. Grade 2 adhesion (Post-operative 5th day)

Table 1. Mazuji scorings for adhesion of all groups after their sacrifices

Groups	Number of animals	Adhesion grades					Adhesion percentages
		0	1	2	3	4	
5 th day SprayShield	8	3	2	1	1	1	62.5%
5 th day seprafilm	8	2	2	3	0	1	75%
5 th day sepracoat	8	3	1	3	1	0	50%
5 th day control	8	2	1	1	3	1	62.5%
14 th day SprayShield	8	2	1	2	2	1	75%
14 th day seprafilm	8	4	2	1	1	0	50%
14 th day sepracoat	8	4	3	1	0	0	50%
14 th day control	8	4	2	1	1	0	50%

There is a wealth of studies which investigated the effectiveness of HA, CMC and HA-CMC in the prevention of adhesions. Reijnen et al.¹⁷, in their rat model of generalized bacterial peritonitis, reported decreased adhesion with HA when compared to saline. In a similar study, HA-CMC was shown to prevent adhesion to some extent in experimental models of rat peritoneum damage; however, CMC alone was found to promote adhesions.¹⁸ In a study focused on a different surgical location, Takeuchi et al.¹⁹ reported anti-adhesive effectiveness of HA-CMC film around rabbit conjunctiva and sclera after surgery. Irkorucu et al.³ reported significant effect of HA-CMC on intra-abdominal adhesions in rats

Table 2. Statistical analysis according to Mazuji classification for the rats sacrificed at 5th and 14th days

5 th day result	Kruskal-Wallis multiple-comparison Z value test			
	1	2	3	4
1	0.0000	0.9054	0.1783	1.0563
2	0.9054	0.0000	0.7270	0.1509
3	0.1783	0.7270	0.0000	0.8779
4	1.0563	0.1509	0.8779	0.0000
14 th day results				
	1	2	3	4
1	0.0000	0.3097	1.8022	0.3097
2	0.3097	0.0000	1.4924	0.0000
3	1.8022	1.4924	0.0000	1.4924
4	0.3097	0.0000	1.4924	0.0000

Regular test: If Z-value >1.9600, medians are significantly different
Bonferroni test: If Z-value >2.6383, medians are significantly different

Table 3. Inflammation scores of rats sacrificed at 14th day

14 th day inflammation scores	Animal number	1	2	3	4	5	6	7	8
	SprayShield	2	2	2	1	2	2	2	2
	Seprafilm	2	2	1	1	0	3	1	1
	Sepracoa	1	1	1	1	1	1	1	1
	Control	1	1	1	1	1	1	2	1
p=0.006 (p<0.05)									
14 th day inflammation	Group	n			Mean rank				
	1	8			24.63				
	2	8			16.50				
	3	8			11.50				
	4	8			13.38				
Total=32									

and did not observe any grade 3 or 4 adhesions. However, they concluded that HA-CMC was insufficient due to poor distribution in abdominal cavity, which caused development of adhesions in areas not covered by HA-CMC. Furthermore, when HA-CMC was applied in areas with infection, it had no positive effect; possibly due to triggering foreign body reaction resulting in increased inflammation.^{3,17,20} Emre et al.²¹ compared honey with HA-CMC in rat cecal abrasion experimental model and surprisingly showed similar effectivity. In another comparison study, Detchev et al.²² showed no superiority of hyaluronate gel on prevention of adhesions compared to serum physiologique. Furthermore, some investigators reported that HA-CMC had no effect whatsoever in preventing intra-abdominal adhesions.^{23,24}

In a large human study comprised of 249 patients who underwent surgery for thoracic outlet syndrome by Sanders et al.²⁵, HA-CMC was applied on the brachial plexus of patients. They reported that HA-CMC eased re-operation by decreasing scarring between the brachial plexus and scalene fat tissue. However, the scarring around neural radices was not reduced and this attempt was accepted as a failure.

Various studies investigating other methods of adhesion prevention exist. A study by Metwally et al.²⁶ which evaluated commonly used anti-adhesives, showed that there is no evidence for the use of steroids, icodextrin, spraygel, and dextran as anti-adhesive agents. Sikkink et al.²⁷, reported that ACP Gel has no effect in decreasing adhesions and abscesses in rat peritonitis experimental model. They also reported it is still a problem to prevent adhesions when contamination is an issue and using an optimal amount of agent with proper viscosity is crucial for the prevention of adhesion. Zong et al.²⁸ reported their findings with a cecal abrasion model. They found that nonwoven PLGA membranes reduced adhesions but the difference was not significant (78% in controls vs.

Table 4. Statistical analysis of all groups in terms of zone calculations

	Groups	n	Mean rank	Standard deviation (micron meter square)
5 th day zone calculations p=0.867 (<0.05)	SprayShield	8	15.50	3635749.09
	Seprafilm	8	16.25	3162452.97
	Sepracoa	8	18.88	276710.77
	Control	8	15.38	992844.29
	Total=32			
14 th day zone calculations p=0.005 (<0.05)	SprayShield	8	26.00	1089704.06
	Seprafilm	8	13.88	786294.11
	Sepracoa	8	16.25	418266.87
	Control	8	9.88	238510.85
	Total=32			

50% with PLGA). However, when PEG-poly(lactic acid) (PLA) was woven into the PLGA membranes, the decrease was significant (78% in controls vs. 22% with PLGA/PEG-PLA). Furthermore, they also were able to impregnate the PLGA/PEG-PLA membrane with the antibiotic Cefoxitin sodium; which reduced the number of adhesions to zero. van Goor et al.²⁹, reported no effect of methyl hydroxyl propyl cellulose gel on adhesion and abscess formation in rats with intraperitoneal infection. In a study by Akdeniz et al.³⁰ it was reported that taurolin irrigation decreased adhesions due to alteration of the fibrinolytic activity of peritoneum; however, there was no significant difference in adhesion score when compared with saline infusion.

Drawing from these very inconsistent and conflicting findings, we believe that the problem with adhesion evaluation is the lack of an objective method to determine the presence and degree of adhesions. Thus, there is a need for an objective evaluation method to replace the subjective analysis of surgeons and the semi-quantitative methods used by pathologists. We believe the “Zone Calculation” method is an objective method which can produce more reliable comparisons between anti-adhesive agents.

In the present study, the PEG-L group was found to have higher adhesion zone score when compared to other materials with the Zone Calculation method (for the 14th-day sacrifice group), whereas HA-CMC and HA group had similar inflammation and adhesion zone scores. The conventional evaluations showed a difference in only the inflammation score of the same PEG-L group, which is suggestive of worse outcome; however, the change of only one parameter can be considered as an inconclusive result. To conclude, we believe that the “Zone Calculation” method is an objective method of adhesion evaluation which can replace conventional methods and provide a standardization for studies focusing on the comparison of anti-adhesive agents.

Ethics

Ethics Committee Approval: The study was approved by the İstanbul University Local Ethics Committee (approval number: 2011/54).

Informed Consent: Not applicable.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.H., Concept: E.H., T.A., Design: E.H., S.E., T.A., Data Collection or Processing: E.H., A.E.A., Analysis or Interpretation: E.H., S.E., T.A., Z.K., Literature Search: E.H., A.E.A., S.D., Writing: E.H., A.E.A., A.O., S.D.

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