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Evaluation of a gold nanocomposite hyaluronic acid-based adhesion barrier with antibacterial properties in an animal model

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ABSTRACT

Objectives: In this study, the effects of a gold nanocomposite hyaluronic acid-based adhesion barrier were evaluated in an animal model.

Materials and methods: In our study, a total of 42 rats in seven groups, with six rats in each group, were evaluated. The groups were established according to the application of an adhesion barrier. In the first, second, and third groups, an adhesion barrier was applied by standard median laparotomy in the first, second, and fourth weeks, respectively. The fourth, fifth, and sixth groups underwent the same procedure in the first, second, and fourth weeks; however, no adhesion barrier was applied to these groups. The seventh group was the control group, and no treatment was performed in this group.

Results: There was no significant difference in the formation of inflammatory cells and fibrous tissue between the groups that underwent laparotomy in the first and second weeks with and without the adhesion barrier ($p>0.05$). However, both low inflammatory cells ($p<0.05$) and low fibrous tissue ($p<0.05$) were evaluated in favor of the adhesion barrier group operated at the fourth week.

Conclusion: A gold nanocomposite hyaluronic acid-based adhesion barrier prevents adhesion, particularly in the long term. However, the results need to be supported by clinical studies.

Keywords: Animal, hyaluronic acid, models, surgery-induced tissue adhesions, surgical adhesions.

Adhesions after surgery that require the opening of the peritoneum can often cause significant and distressing results. The incidence of adhesion in surgical procedures in which the abdomen is opened is up to 90%, and in gynecological procedures where the pelvis is opened, it is up to 97%.[1,2] These procedures can induce a broad range of issues, such as infertility, abdominal and pelvic pain, bowel obstruction, and difficulties experienced during reoperative interventions.[3-8] Postoperative adhesions most commonly occur in the early postoperative period. After surgical trauma or other damaging conditions, the inflammatory cascade is triggered, increasing fibrin in the damaged area.[9] Many different materials and medical agents have been produced to prevent adhesions. Some of these products are in the form of membranes, while others are in the form of gel barriers.[10-12] In a study comparing a hyaluronic acid gel and a hyaluronic acid carboxymethylcellulose product, it was demonstrated that the application of hyaluronic acid gel reduced the number of organs undergoing adhesion but did not cause a significant reduction in the degree of adhesion.[13]

In this study, the histopathological effects of a gold nanocomposite hyaluronic acid-based adhesion barrier, designed as a new type of gel barrier, were evaluated on an animal model.

MATERIALS AND METHODS

Animals and experiment method

The study was conducted on a total of 42 Wistar albino rats obtained from the SYLAB Experimental

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Animal Laboratory in seven groups, with six rats in each group. Each group included three adult males weighing 275±10 g (range, 255 to 300 g) and three adult females weighing 225±18 g (range, 200 to 250 g). The rats were kept in cages of equal size for a maximum of four weeks according to the study groups at a constant temperature of 20°C and in a 40-55% humidity laboratory environment on a 12-h daylight, 12-h night cycle. Standard rat chow was used for rats in all groups (25 g/day). The waters of the rats in all groups were changed every other day. At the end of the experiment, all animals were sacrificed by administering a high-dose anesthetic. The rats were cared for and fed using the facilities in the experimental animal laboratory of Sivas Cumhuriyet University.

Control group, and no treatment was performed on the animals. The groups were established according to the application of an adhesion barrier. In the first, second, and third groups, an adhesion barrier was applied in the first, second, and fourth weeks, respectively. The adhesion barrier was applied by standard median laparotomy in these groups. The fourth, fifth, and sixth groups underwent the same standard median laparotomy procedure in the first, second, and fourth weeks; however, no adhesion barrier was applied to these groups. The seventh group was the control group, and no treatment was performed in this group.

After general anesthesia (subcutaneous ketamine 87 mg/kg and intraperitoneal 3 mg/kg xylazine) was administered, the abdominal region of the rats was shaved. After surgical site sterilization, the skin and subcutaneous tissues were passed, and the abdomen was reached. After reaching the abdomen, the intra-abdominal organs were manually manipulated and with surgical forceps in all groups except for the control group. After the manipulation, 4 mL of adhesion barrier (Metrical Medical Devices Software Defense Industry and Trade Limited Company, Sivas, Türkiye) was applied to each rat in the adhesion barrier groups (Figure 1). The same manipulations were performed in the standard surgical groups, but the adhesion barrier was not applied. After the procedures were completed, subcutaneous closure was performed with 2-0 Vicryl sutures for the subcutaneous tissues and 4-0 polyglactin (Vicryl) sutures for the skin. Each operation was performed by the same surgeon to avoid surgical differences during the procedure. Daily dressing was done until the wounds were completely healed. The rats in each group were sacrificed with a high-dose anesthetic at the designed time in the study. Peritoneal tissue samples were taken after the animals were sacrificed. Extracted specimens were histopathologically evaluated, and comparisons were made for each group.

Histopathological method

Peritoneal samples taken from sacrificed rats were fixed in 10% neutral formalin. Tissues were taken into paraffin blocks after routine alcohol-xylol procedures, and 5 µ sections taken on slides with polylysine were stained with hematoxylin-eosin and Masson's trichrome. Histopathological evaluation was evaluated in terms of edema, vascularization, and inflammatory cell infiltration, similar to the study of Papparella et al.[14] The fibrous tissue thickness formed in staining with Masson's trichrome was measured and classified (Table 1). The scoring systems of Zühlke et

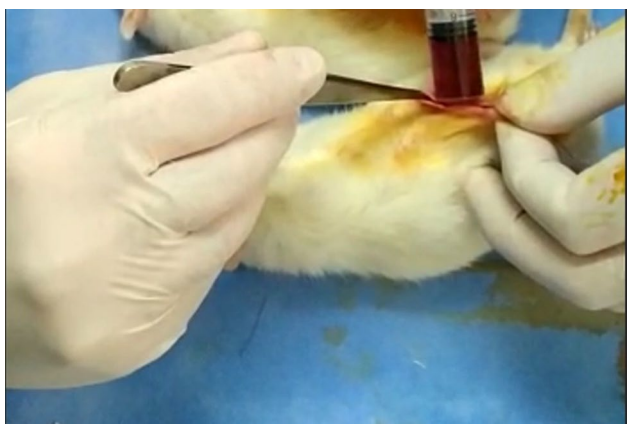

Figure 1. Adhesion barrier application is seen.

Table 1
Histological scoring system[12]

Histopathological scores	
No changes	Absent (-)
Less than 10%	Mild (+)
Between 10-40%	Moderate (++)
More than 40%	Severe (+++)
Fibrous tissue scores	
Less than 20 µm	Absent (-)
20-80 µm	Mild (+)
80-160 µm	Moderate (++)
More than 160 µm	Severe (+++)

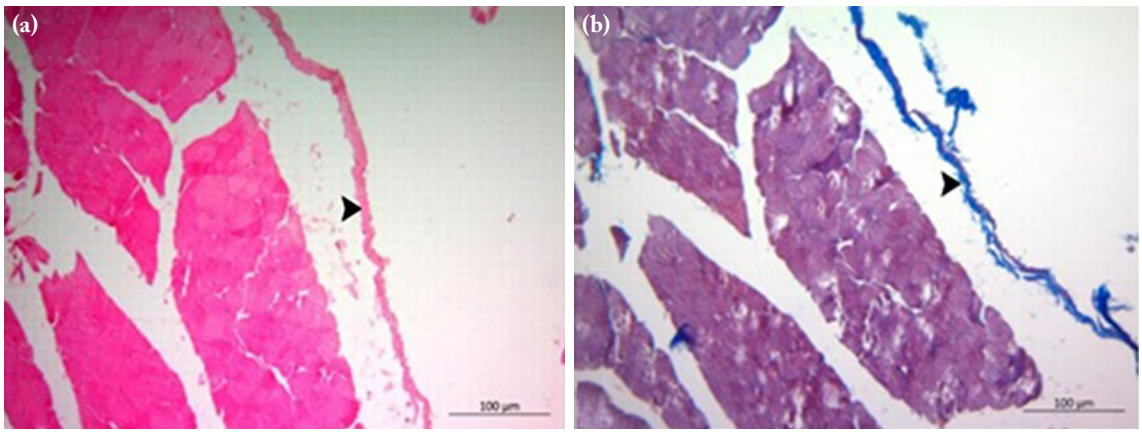

Figure 2. Control group. **(a)** Hematoxylin-Eosin, **(b)** Masson's Trichrome Staining. Normal histological appearance. Mesothelial layer (arrowhead), (×40).

al.[15] and Nair et al.[16] were not used in the study as it was mostly based on observational evaluation in assessing the degree of adhesion. Inflammation (0-3 days, acute), proliferation (3-12 days, subacute), and remodeling (>12 days, chronic) stages used in wound healing were also used in peritoneal wound healing.[17] Histopathological evaluation was based on the study of Kojima et al.[18]

Statistical analysis

The data were analyzed with the IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). The difference between the groups was determined by Student's t-test, which is a nonparametric test. A p value of <0.05 was considered statistically significant.

RESULTS

Peritoneal specimens of rats in the control group had a normal histological appearance (Figure 2). Statistically significant histopathological differences were found between the treatment groups (p<0.05).

Mild edema and inflammatory cell infiltrations were observed in the first and fourth groups. There

Table 2
Histopathological evaluation results.[a,b,c,d] Different letters in the same column indicate statistical difference between groups (p<0.05).[A,B,C] Different letters on the same line indicate statistical difference between groups (p<0.05).

Groups	Edema	Inflammatory cell infiltration	Vascularization
	Mean±SD	Mean±SD	Mean±SD
The group that did not apply an adhesion barrier in the first week.	1.00 ± 0.00^{aA}	0.83 ± 0.40^{aA}	0.12 ± 0.40^{aA}
The group in which an adhesion barrier was applied for the first week.	0.83 ± 0.40^{aA}	0.83 ± 0.40^{aA}	0.33 ± 0.51^{aB}
The group that did not apply an adhesion barrier in the second week.	2.83 ± 0.40^{bA}	2.66 ± 0.51^{bA}	1.83 ± 0.40^{bB}
The group in which an adhesion barrier was applied for the second week.	1.66 ± 0.51^{cA}	1.83 ± 0.40^{cA}	1.00 ± 0.00^{cB}
The group in which no adhesion barrier was applied in the fourth week.	2.66 ± 0.40^{bA}	2.83 ± 0.40^{bA}	2.66 ± 0.51^{dA}
The group in which the adhesion barrier was applied at the fourth week.	1.12 ± 0.40^{aA}	1.12 ± 0.40^{aA}	1.00 ± 0.00^{cA}

SD: Standard deviation.

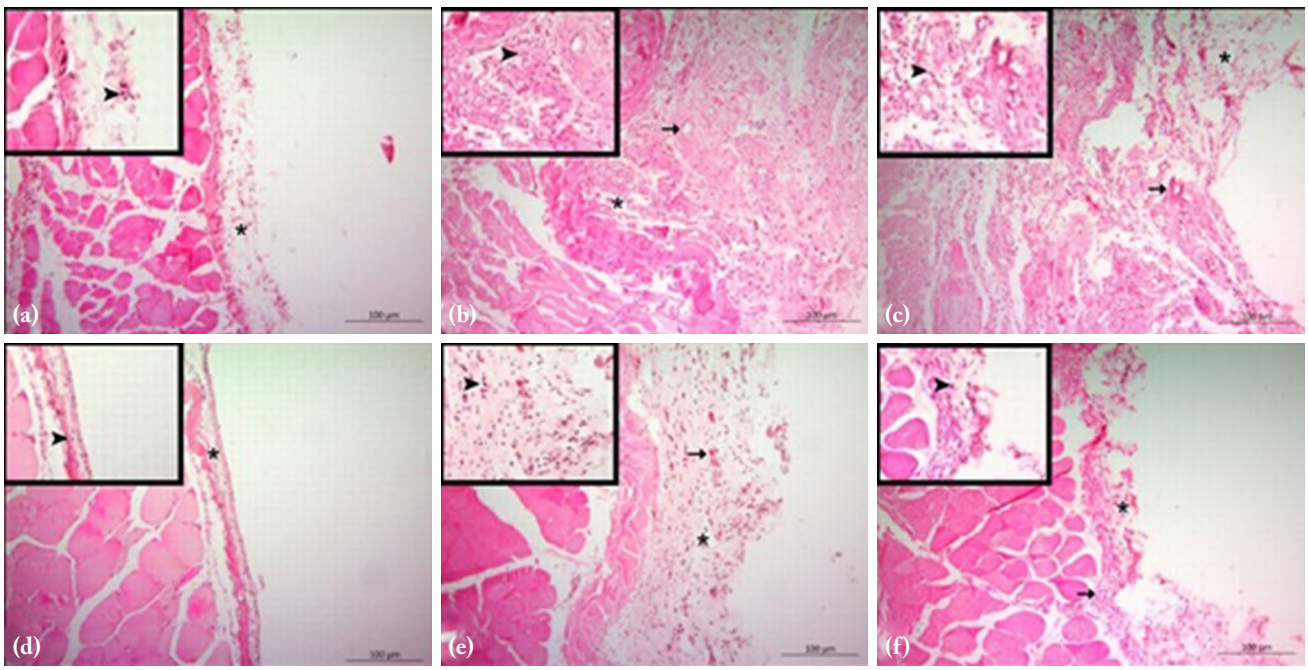

Figure 3. **(a)** The fourth group: Mild edema (*) and inflammatory cell infiltration; **(b)** The fifth group: Severe edema (*), inflammatory cell infiltration (arrowhead) and moderate vascularization (arrow); **(c)** The sixth group: Severe edema (*), inflammatory cell infiltration (arrowhead) and vascularization (arrow); **(d)** The first group: Mild edema (*) and inflammatory cell infiltration; **(e)** The second group: Moderate edema (*), inflammatory cell infiltration (arrowhead) and mild vascularization (arrow); **(f)** The third group: Mild edema (*), inflammatory cell infiltration (arrowhead), and vascularization (arrow), hematoxylin-eosin, (×40).

was no statistically significant difference between these two groups. While severe edema, inflammatory cell infiltration and moderate vascularization were determined in the fifth group, edema and inflammatory cell infiltration were moderate and vascularization was mild in the second group. The most significant histopathological difference was determined at four weeks. While edema, inflammatory cell infiltration, and vascularization were determined as severe in the group without an adhesion barrier at four weeks (the sixth group), these histopathological findings were mild in the third group, in which an adhesion barrier was applied (Table 2, Figure 3).

Statistically significant differences were detected between the groups in fibrous tissue formation on staining with Masson's trichrome. While mild

Table 3
Masson's trichrome staining findings.[a,b,c] Different letters in the same column indicate statistical difference between groups ($p<0.05$)

Groups	Fibrous tissue formation
	Mean±SD
The group that did not apply an adhesion barrier in the first week.	0.83±0.40[a]
The group in which an adhesion barrier was applied for the first week.	0.83±0.40[a]
The group that did not apply an adhesion barrier in the second week.	1.66±0.51[b]
The group in which an adhesion barrier was applied for the second week.	1.83±0.40[b]
The group in which no adhesion barrier was applied in the fourth week.	2.83±0.40[c]
The group in which the adhesion barrier was applied at the fourth week.	1.00±0.00[a]

SD: Standard deviation.

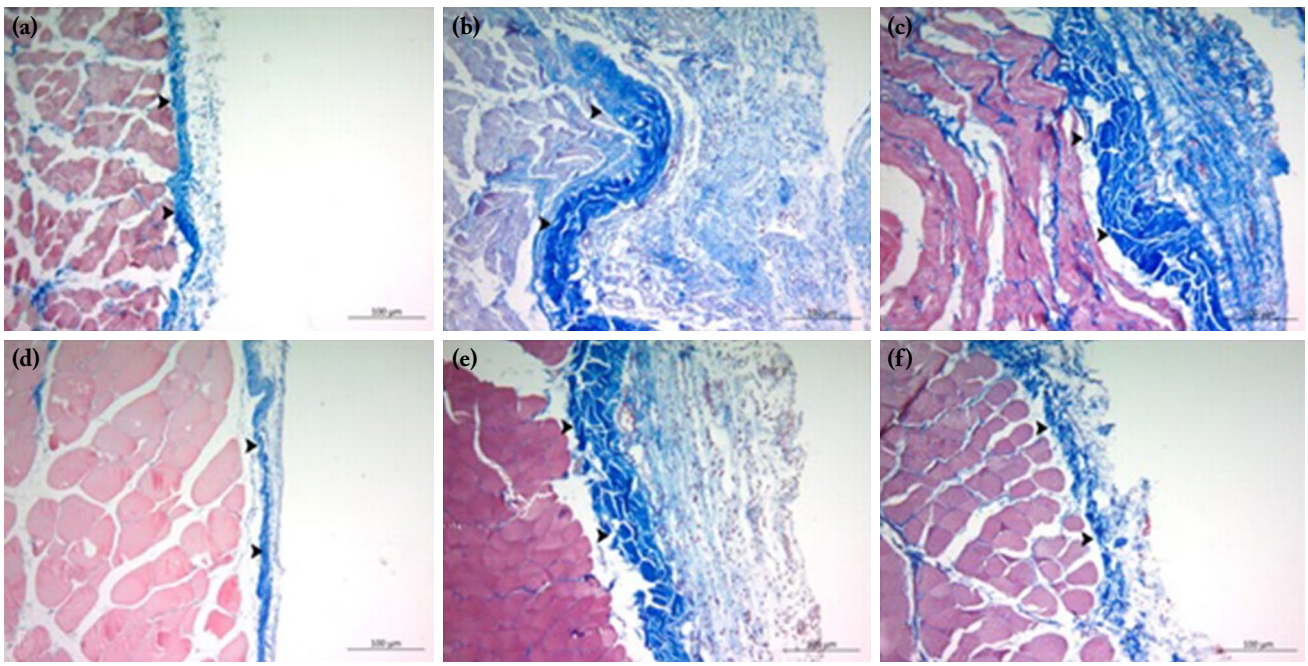

Figure 4. **(a)** The fourth group: Mild fibrous tissue formation (arrowhead); **(b)** The fifth group: Moderate fibrous tissue formation (arrowhead); **(c)** The sixth group: Severe; **(d)** The first group: Mild fibrous tissue formation (arrowhead); **(e)** The second group: Moderate fibrous tissue formation (arrowhead); **(f)** The third group: Mild fibrous tissue formation (arrowhead); Masson's trichrome, (×40).

fibrous tissue formation was detected in the first and fourth groups, moderate fibrous tissue formation was detected in both these groups. There was a significant difference between the third and sixth groups. While severe fibrous tissue formation was observed in the sixth group, mild fibrous tissue formation was observed in the third group (Table 3, Figure 4).

DISCUSSION

Postoperative adhesions are considered a risk factor for redo surgeries.[19] After surgeries in which the solid organs are not covered by the peritoneum or pericardium, adhesions from the previous surgery increase the complexity of the surgical procedure and are associated with increased mortality/morbidity when a new surgery is required. Adhesions seen after any surgery are one of the most important factors affecting the course of redo abdominal surgery.[20,21] The main mechanism of adhesion formation is the migration of inflammatory cells to the surgical site in the acute and chronic phases. Essentially, this migration takes place to speed up recovery, but when surgery is required again, it complicates the surgical process of the patient. Our study was designed based on abdominal adhesions.

Adhesion barriers are currently used to eliminate or minimize the risk of postsurgical adhesion. These barriers prevent the inflammatory cascade or fibrin formation and form a mechanical barrier by preventing the approach and contact between the affected tissues that cause adhesion formation. Abdominal adhesions are associated with significant comorbidities, such as chronic pelvic pain, dyspareunia, infertility, and intestinal obstruction. Adhesions can also cause issues in other specialties, such as gynecology, oncology, or pediatric surgery. There are large financial and public health repercussions associated with hospital readmission costs, and they represent a real public health problem.

There are many products produced to prevent adhesions.[19,22] It has been shown that the use of polyethylene glycol/polylactic acid membrane containing barriers, alone or with other barriers, prevent adhesion to a significant extent.[23] However, polylactide film barrier was found to be ineffective in preventing adhesions.[24] An ideal adhesion barrier

prevents the formation of adhesions by allowing the damaged tissue surfaces to separate and heal freely. In addition, the barrier must be nonreactive, antibacterial, biocompatible, biodegradable, and effective *in vivo*.[20,21] In this study, gold nanoparticle/hyaluronic acid nanocomposite was synthesized *in situ* without the use of toxic chemicals and the purification step by green synthesis.

This study evaluated the effectiveness of the adhesion barrier, mainly targeting the acute, subacute, and chronic processes. When the acute period effects were examined, no significant difference was observed between the groups with and without the application of the adhesion barrier in terms of both the density of inflammatory cells and the formation of fibrous tissue. As the process lengthened and the subacute and chronic periods were reached, there was a significant decrease in inflammatory cell and fibrous tissue density in favor of the adhesion barrier group. Furthermore, in terms of edema and vascularization, there was a significant decrease in favor of the adhesion barrier group.

It was shown that the application of an adhesion barrier prevents cellular activities that will cause adhesion in rats. This is promising for patients who will need surgery again. These findings suggest that a gold nanocomposite hyaluronic acid-based adhesion barrier can be successfully applied to prevent postsurgical adhesions. However, clinical studies with long-term follow-up are needed.

There are several limitations in our study. The first of these only conducted research on the The research was only conducted on the laparotomic approach. In addition, the study being an animal experiment limited the chance of long-term follow-up. The study was only tested on peritoneal adhesions. Further studies are needed to investigate its effects on other membranes, such as the pericardium.

In conclusion, the application of the adhesion barrier will cause adhesions in the subacute and chronic periods. It was observed that the adhesion barrier minimizes inflammatory cells, edema, vascularity, and fibrous tissue formation. In terms of these parameters, no statistically significant difference was observed between the two groups in the early period, suggesting that the antibacterial gold nanocomposite hyaluronic acid-based adhesion barrier can be successfully applied to prevent subacute and chronic adhesions. Further clinical studies with long-term follow-up are needed.

Ethics Committee Approval: The study was initiated after the ethics committee decision numbered 65202830-050.04.04-524 of Sivas Cumhuriyet University Animal Experiments Local Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, critical review: F.K.; Control/supervision, data collection, literature review, writing the article, critical review, references: F.A.; Analysis and/or interpretation, literature review: M.Ö.; Analysis and/or interpretation: A.S.M.

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