

# Experimental Adhesion Model: Effect of Viscosities of Fluids Put in the Peritoneal Cavity on Preventing Peritoneal Adhesions

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**Abstract:** In this study we assessed the effectiveness of fluid viscosities placed in the peritoneal cavity to prevent postoperative peritoneal adhesions. Thirty-six Wistar albino female rats (average weight:  $160 \pm 30$  g, average age: 6.5 months) were divided into three groups of equal number. A standard adhesion pattern was formed in each group. Then, 3 ml isotonic sodium chloride solution (relative viscosity value: 1) was added into the peritoneal cavity of group 1; 3 ml standard 6% hydroxy ethyl starch solution (HES) (relative viscosity value: 2.9) was added into the peritoneal cavity of group 2; and a standard HES solution that was concentrated by dehydration (relative viscosity value: 249.7) was added into the peritoneal cavity of group 3. All rats were sacrificed on postoperative day 10 and the adhesions that formed were graded. In group 1, grade-3 adhesions developed in 9 (75%) rats, and grade-2 developed in 3 (25%) rats. In group 2, grade-3 adhesions developed in 1 (8.3%) rat, grade-2 developed in 6 (50%) rats, and grade-1 developed in 5 (41.6%) rats; in group 3, grade-3 adhesions developed in 9 (75%) rats, and grade-2 developed in 3 (25%) rats. The adhesion scores of group 3 and group 1 were equal to each other ( $P=1$ ), while the adhesion score of group 2 was significantly less ( $\chi^2:18.23$ ,  $P<0.001$ ). Increasing the viscosity of fluids that are inserted in the peritoneal cavity may reduce the formation of postoperative peritoneal adhesions till a critical value of unknown viscosity is achieved. The mechanism behind this process remains unclear.

**Key words:** adhesion, fluid, periton, prevention, viscosity

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## Introduction

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Postoperative peritoneal adhesions (PPA) constitute a serious problem in abdominal surgery. PPA occur in nearly more than 90% of laparotomy cases and intesti-

nal obstruction develops in 3% of cases [5, 15]. Furthermore the cause of female infertility in almost 15–20% of cases is PPA [1, 11]. Various substances and/or techniques are used to solve the problem, but unfortunately an effective solution has not yet been

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achieved [5]. One of the most frequent methods that is used to prevent PPA is to put various fluids in the peritoneum.

The internal strength that resists the flow of a certain fluid is recognized as viscosity. In this study we investigated a hypothesis suggesting that late absorption of fluids with a high viscosity, and formation of an interface layer preventing the contact of surfaces would be effective for prevention of PPA formation. In vitro studies have displayed that, a cell or a group of cells located at the both sides of a medium with a high viscosity may demonstrate a delayed motion against each other, therefore, adhesions do not occur due to the level of viscosity or the adhesion process is significantly inhibited by it [8].

In the present study, we investigated the relationship between viscosity and its anti-adhesive effect by placing different viscosities of the same fluid into the peritoneal cavity.

## Materials and Methods

### Preliminary study

During the study, a 6% hydroxy ethyl starch (HES) suspension (Plasma Sterile®, Fresenius Kabi Co) with a molecular weight of 450,000 Dalton and an osmosis of 309 mOsm/l was used in the peritoneal cavity. The 1,000 ml HES suspension was dehydrated till the suspension was 150 ml and a more viscous form of the HES suspension (vHES) was obtained.

To determine the relative viscosities of fluids used in the study an "Oswald Relative Viscosity Assay" was performed [10]. Three plastic tubes with a 3 mm internal diameter and 1 meter in length were positioned upright and a tap was fitted to their lower outlet. Each of the tubes was filled with one of the study fluids and a metallic sphere that weighed 1 g with an external diameter of 1 mm was inserted into each of the tubes. The time taken for the sphere move down the entire tube was measured. This procedure was repeated six times for each tube and the mean time was calculated for each group. Finally, the mean relative viscosities of the fluids were determined (Table 1).

### Method

Consent was received from the Istanbul University, Cerrahpasa Faculty of Medicine, Experimental Animals

**Table 1.** Oswald relative viscosity assay results

Group	Mean Sphere Moving Time (s)	Mean Relative Viscosity (0.9%NaCl Related)
1	6.3	1.0
2	18.6	2.9
3	1573.3	249.7

Production and Research Laboratory Ethical Committee. The number of animal subjects was determined after negotiation with a biostatistician specialist. Thirty-six Wistar albino female rats (average weight  $160 \pm 30$  g, age: 5–7 months, average age: 6.5 months) were selected for the study. Rats were kept in plastic based production cages with plastic sides which were covered by a wire knitted cover placed over the top of the cage and animals were fed by privately manufactured pellet type factory fodders prepared especially for such animals.

Rats were divided into three groups of equal number and after one night of fasting they were anesthetized in jars with ether for a period of 45–60 s. Anesthesia was maintained with an injection of 75 mg/kg intramuscular Ketamin (Ketalar®, Eczacibasi Co).

The middle line of the abdomen where the incision was performed was carefully shaved and then antiseptis was provided by povidon iodine application. After a 3 cm vertical midline incision the peritoneal cavity was entered. The caecum and the terminal ileum were found and covered with a damp piece of gauze. Scraping was applied to the caecum and the 2 cm terminal ileum with a piece of dry gauze. This process was continued until superficial petechial bleeding lesions were made (The Scraping Model) [4].

The above procedures were applied to all rats in the same manner. After completion of these procedures, 3 ml 0.9% NaCl suspension was added into the peritoneal cavity of the rats in group 1, 3 ml HES suspension was added into the peritoneal cavity of the rats in group 2, and 3 ml vHES suspension was added into the peritoneal cavity of the rats in group 3. The incision was closed by a continuous suture technique using 000 polypropylene. After the postoperative 24th h, rats were fed by usual feeding procedures.

All rats were sacrificed on the postoperative 10 day by prolonged ether inhalation (minimum 10 min). To view the entire adhesions and to perform correct grad-

ing the peritoneal cavity was entered with a “reversed U” incision. The abdominal front wall flap was drawn towards the caudal without causing any damage to the adhesions. Adhesions were graded as 0, 1, 2, 3, compatible with the Evans model (Table 2) [7]. The sacrifices and repeat laparotomies were performed by the authors of this study but the evaluation of adhesions was performed by a clinical resident of who was unaware of the groups of rats and the exact procedure of this study.

#### *Morphologic evaluation*

Terminal ileum and ceacum were excised with their adhesions. Pieces were fixed in 70% alcohol. They were immersed in paraffin after dehydration. Sections of 5  $\mu\text{m}$  thickness from the paraffin molds were taken, stained with hematoxylin and eosin, and examined under a microscope.

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### Results

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Statistical evaluation was performed with the aid of a GraphPad Prisma V.s3 program and the data was assessed by the means of Chi-Square test. Results were assessed at a 95% confidence limit and the “*P*” value was interpreted as significant when less than 0.05 and was interpreted as highly significant when less than 0.001.

The adhesion scores of groups and statistical evaluation are shown in Table 3. Group 3 and group 1 adhesion scores were equal ( $P=1$ ); however the mean adhesion score of group 2 was highly significantly lower than those of group 1 and 3 ( $\chi^2:18.23, P<0.001$ ).

A histopathologic examination was performed under  $\times 100$  magnification, light microscope. In group 2, fibrinous exudates in the peritoneal surfaces, serosal thickening, edema, nonspecific inflammatory cell infiltration, capillary proliferation and micro abscess foci were observed. In group 3 and group 1, fibrinous exudates in the peritoneal surfaces and serosal edema were much greater. Inflammatory cells were composed of especially histiocytes; micro abscess foci and capillary proliferation were observed as in group 2 (Figs. 1 and 2).

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### Discussion

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The internal strength that resists the flow of a fluid is

**Table 2.** Evans model

Adhesion Grade	Definition
0	No adhesion
1	Spontaneously separating adhesions
2	Adhesions separating by traction
3	Adhesions separating by dissection

known as viscosity. A fluid is accepted viscous according to its fluidity. A fluid with a slower fluidity is considered more viscous. There are three factors that have an impact on viscosity: temperature, density of the fluid and the medium pressure. All of these three factors are directly related to viscosity. In other words, if the temperature of the fluid and/or the density of the fluid and/or the pressure of the fluid increases, viscosity will also increase. When these factors tend to decrease so does viscosity [10, 18]. In the present study we heated all the fluids in an autoclave up to 36°C (body temperature) and prior to each assay we washed the test tube with 0.9% NaCl at 36°C.

The viscosity unit is Newton-Second per meter square when the SI system is used ( $\text{N}\cdot\text{s}/\text{m}^2$ ). Another frequently used unit is Poise (P); 1 P, is equal to the 1/10 of 1  $\text{N}\cdot\text{s}/\text{m}^2$  [10].

The specific viscosity of a fluid can be calculated by using the following formula:

$$n = 2(\Delta p) \cdot g \cdot a^2 / 9v$$

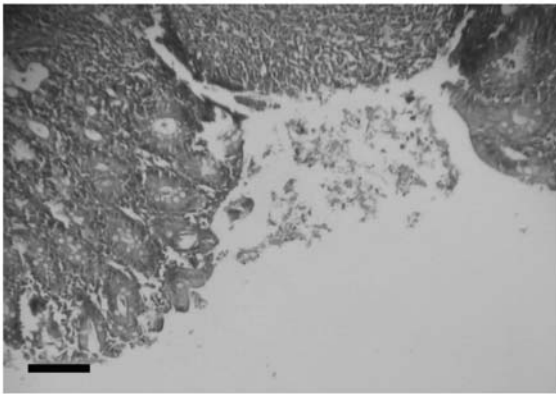
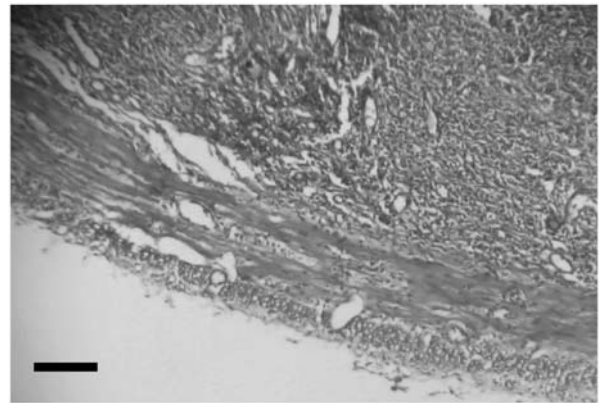
( $\Delta p$ : differences between sphere and fluid densities,  $g$ : gravity acceleration,  $a$ : diameter of sphere,  $v$ : falling speed of the sphere in the fluid)

Various complex devices called viscometer have been developed for this purpose. With these devices it is now easy to determine the relative viscosities of fluids. The most frequent method of viscosity measurement that is used is the “Oswald Relative Viscosity Assay”. We described it in detail in a preliminary study, and this assay is used to reveal multiple viscosity differences of fluids relative to each other [10].

To prevent the formation of PPA, many substances which react by different mechanisms have been tested [6]. One effect of the mechanisms of these substances is to form a mechanical barrier between the peritoneal surfaces. To realize this goal, various substances have been added into the peritoneal cavity for testing. Some of these substances were non-absorbable solid materials (cattle peritoneum, amnion membrane etc) and others

**Table 3.** Adhesion grades of groups and statistical evaluation results

Adhesion Grade	Group-1	Group-2	Group-3	Statistical Evaluation (Chi-Square Test)
1	0 (0%)	5 (41.7%)	0 (0%)	$\chi^2$ :11.61 $P=0.003$
2	3 (25%)	6 (50%)	3 (25%)	$\chi^2$ :2.25 $P=0.32$
3	9 (75%)	1 (8.3%)	9 (75%)	$\chi^2$ :14.26 $P=0.0008$
Total	12 (100%)	12 (100%)	12 (100%)	-

**Fig. 1.** Light micrograph of adhesion area showing nonspecific inflammatory cell infiltration, ulceration and capillary proliferation. Hematoxylin and eosin. Magnification: 100 $\times$ . Bar = 40  $\mu$ m.**Fig. 2.** Light micrograph of adhesion area showing nonspecific inflammatory cell infiltration, fibrinous exudates and serosal edema. Hematoxylin and eosin. Magnification: 100 $\times$ . Bar = 40  $\mu$ m.

were absorbable solid materials (hyaluronic acid derivatives, oxidized celluloses) which become liquidized at body temperature. Liquid substances such as olive oil, soybean oil, liquids made from glucose, starch and glycerol have also been used [2, 3, 17].

Fundamentally, all these substances are intended to mimic the activity of peritoneal physiological mechanisms. One of the natural anti-adhesive mechanisms included in the intact peritoneal cavity is a viscous fluid that is assumed to be secreted from the mesothelial cells, between the peritoneal surfaces. In our study we planned to imitate this natural mechanism.

To achieve our goal we knew that the best fluid should not damage the restoration process of the peritoneal membrane which had been damaged and should be viscous enough to avoid contact of the damaged surface with other surfaces. In addition, the fluid had to remain within the peritoneal cavity without it becoming absorbed for a period of few days. Because peritoneal damage always develops after a trauma that

impairs the integrity of the peritoneum, PPA form within 5–7 days after the trauma [12]. According to our hypothesis, high viscous fluids would be difficult to absorb by the peritoneal lymphatic system. Accordingly, a fluid that remained in the peritoneal cavity for a long period would prevent the contact of damaged peritoneal surfaces with each other and therefore the adhesion of such surfaces with each other would also be prevented.

To investigate the importance of viscosity in preventing the formation of PPA we tried to examine a wide fluid scale and carry out an objective assessment. The fluids needed to have the same physical properties, but significantly different viscosities. There are no significant viscosity differences among proteolytic agents, fibrinolytic materials, steroids and heparins. Besides, the substances with similar physical properties that are listed under this category are few in number. Oils have a high viscosity. Furthermore, there are a number of oils that possess a different value of viscosity. How-

ever, identical oils do not have different viscosities. Therefore, it is not convenient to test different oils with a different viscosity due to their different physical properties. In the present study we chose to use colloidal fluids. Because colloidal fluids contain larger molecules than crystalloid fluids and are more viscous, and their viscosity can be increased simply by dehydration.

For this purpose we chose to use HES fluid. We dehydrated 1,000 ml original HES and reduced its volume to 150 ml. This way we succeeded in increasing its original viscosity 84.5 fold while also increasing its viscosity relative to the control fluid, 0.9% NaCl, 249.7 fold.

Different classifications can be used to perform the grading of PPA [9, 13, 14, 16]. Graded adhesions described by Evans are classified according to the adhesion intensity to seroserosal surfaces and are described as 0, 1, 2 and 3 [7]. We assume that the impact of the intensity of this adhesion plays a significant role in the development of complications due to PPA. Due to this and the practical application, we performed the adhesion grading process in our study in accordance with the Evans model. When compared with group 1 (the control group), HES highly significantly reduced the PPA in group 2 rats ( $P < 0.001$ ), but there was no difference between the Evans grade of group 3 (vHES) and group 1 (the control group).

Two important questions are raised by this result, 1) Is there a critical value related with viscosity increase? Or, in order to prevent PPA, what benefits are provided at what viscosity of the fluid placed in the peritoneal cavity, and beyond that what value of viscosity do the benefits begin to disappear? To respond to this question we assume that new experimental studies are required to rank the benefits with the viscosity values of the same fluid. 2) What mechanism allows to an increase in viscosity at a certain level reacts ineffective as 0.9% NaCl on the PPA formation? If we consider that there is no evidence to explain the mechanism, then we assume that, we must suggest that the response is probably related to the peritoneal lymphatic process. Because macromolecular substances such as starch cannot be absorbed by the peritoneal capillary vessels, there must be a peritoneal lymphatic system that is responsible for their removal from the peritoneal cavity.

The vHES fluid contains a large number of macro molecules and therefore its absorption period can de-

crease and even canals can become obstructed. Fluids which remain in the peritoneal cavity for a long period may form foreign substances and trigger the formation of PPA.

Consequently, increasing the viscosity of fluids that are added into the peritoneal cavity may somehow reduce the formation of PPA till a critical value of an unknown viscosity is reached. However the mechanism of this process remains unclear.

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