

# PERIDAN™ Film Safely Inhibits Postsurgical Adhesions in a Rat Model

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## INTRODUCTION AND METHODS

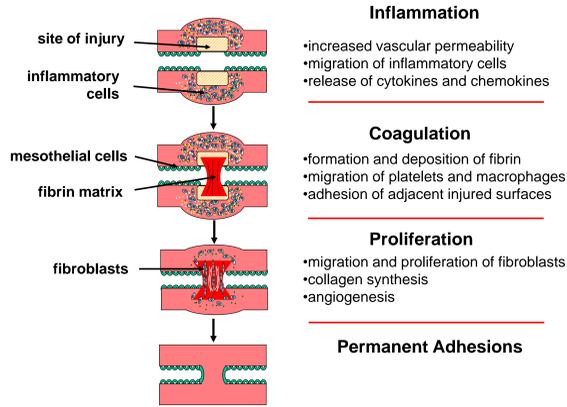
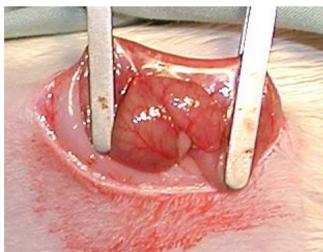


Figure 1: Diagram illustrating the main steps in adhesion formation.

Adhesions form when serosal surfaces are traumatized resulting in an inflammatory reaction with release of cytokines, growth factors and angiogenic factors from activated macrophages, neutrophils, and other inflammatory cells. The presence of a fibrin-rich exudate, coupled with a decrease in peritoneal fibrinolytic capacity leads to the development of a fibrin matrix, which provides a scaffold for the adherence and proliferation of fibroblasts, mesothelial cells, and endothelial cells. As tissue remodeling proceeds, cell differentiation and growth, extracellular matrix deposition and angiogenesis occur, resulting in the formation of dense, vascularized fibrous bands. Therapeutic approaches to adhesion prevention have included both pharmacological and physical barrier methods. Numerous pharmacological agents have been evaluated for their anti-adhesion abilities, each targeted towards attenuation of a specific pathological mechanism implicated in the adhesiogenic process. Although some of these drugs demonstrated initial efficacy in a limited number of animal models, none have shown adhesion-reduction benefits in the clinical setting and many have exhibited deleterious side effects. In addition to appropriate surgical technique the primary clinical strategy for adhesion prophylaxis is the mechanical separation of peritoneal surfaces by the use of barrier systems. These barriers exist in liquid, viscous gel, and solid form. The crucial mode of action of these products is prevention of tissue apposition, which in turn minimizes the formation of fibrin attachments between surgically traumatized tissue surfaces, a recognized first step in adhesion formation. The objectives of this work were to evaluate the efficacy of various compounds loaded into biodegradable sodium hyaluronate (HA) carrier films for prevention of post-surgical adhesions and to further evaluate the most promising candidate in load-limiting efficacy and toxicity studies. The results from local administration during caecal-sidewall surgery in rats are presented.



The abdominal wall was retracted to permit excision of the peritoneum. A single suture was made at each of the lower corners of the abraded area to the abraded caecum. The gel was placed between the abraded area and the caecum.



The upper margin of the caecum was then attached to the upper border of the abraded area by two corner sutures.

Figure 2. Surgical procedure used in the rat caecal-sidewall model for surgical adhesion. The caecum and sidewall were injured and stitched together. Seven days post-surgery the animals were sacrificed for adhesion assessment. The adhesion strength was scored from 0 (no adhesions) to 3 (dense adhesions with unavoidable tearing of tissue). The extent of adhesion formation was quantified by assigning a value of 1 to 4 according to the total area of the peritoneal defect involved. A final adhesion score for the caecum-sidewall area was then calculated by multiplying the two numbers. The maximum Adhesion Value in this model is 12.

## RESULTS

A. Of the 11 drugs and 2 polymers examined in this study, only TIMP-2, cisplatin and fucoidan had significantly reduced adhesion scores when compared with control films (Table 1). Fucoidan film significantly reduced adhesion scores compared with all other candidates ( $p < 0.05$ ) and 7/12 animals treated with fucoidan film were adhesion free (Figure 2). Incision thickness, WBC counts and percent changes in initial weight in the fucoidan group were not different from the control film group.

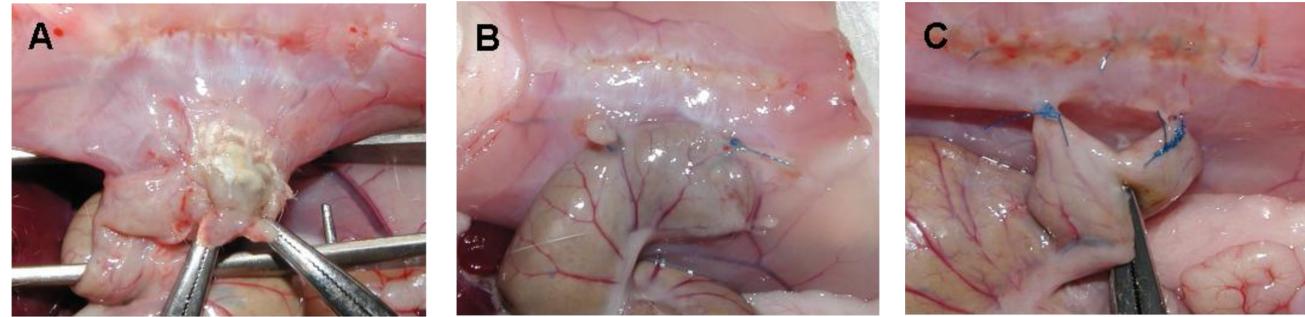


Figure 3: Photographs of the surgical site in rats following caecal-sidewall surgery and (A) no treatment or locally administered (B) control sodium hyaluronate film or (C) fucoidan film. Photographs were taken at time of sacrifice seven days post-surgery. The adhesion scores were 12, 6 and 0, respectively.

B. Further evaluation of fucoidan demonstrated that 0.1, 1 and 10 mg fucoidan per film significantly reduced adhesion scores compared to control films. No statistical difference was found between these fucoidan groups ( $p < 0.05$ ) and 9/12 animals were adhesion free (Figures 2 and 3). No effect of fucoidan film was observed on the incision thickness, WBC counts or the percent change in initial weight over the 7 days of the study (Table 2).

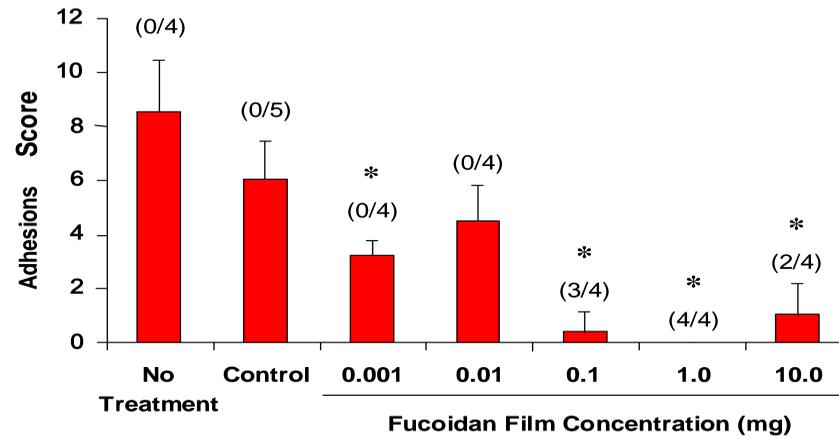


Figure 2: Effect of films loaded with various concentrations of fucoidan on adhesion formation following caecal-sidewall surgery in rats. Each bar represents the mean adhesion score  $\pm$  standard deviation. The fraction above each treatment group represents the number of adhesion free animals over the number of animals in the treatment group. \* statistically significant compared with control film

C. In the toxicity study, no difference in clinical signs, appearance or behavior was noted between rats that received control films or those that were administered fucoidan film (10 mg) during the 4 day study period. No abnormalities were observed in the abdominal cavity at necropsy. A modest but significant increase in hemoglobin and hematocrit values (less than 8% and 6% respectively) at day 1 were noted in the fucoidan film group compared with the control film group (Table 3). No other differences in hematology parameters were observed between the two groups. There were no differences between WBC counts obtained prior to surgery and at time of sacrifice within any group. Differential blood analysis showed no difference between the two groups (Table 4).

Table 3: Effect of fucoidan film on complete blood count following caecal-sidewall surgery in rats. Data represent the range of 3 rats per group.

| Parameter                          | Control Film |         |         |         | Fucoidan Film |         |         |         |
|------------------------------------|--------------|---------|---------|---------|---------------|---------|---------|---------|
|                                    | Day 1        | Day 2   | Day 3   | Day 4   | Day 1         | Day 2   | Day 3   | Day 4   |
| WBC ( $\times 10^3/L$ )            | 1.0-1.3      | 0.7-1.1 | 1.1-1.7 | 0.9-1.8 | 1.1-1.4       | 1.0-1.3 | 1.2-1.6 | 0.5-0.9 |
| RBC ( $\times 10^{12}/L$ )         | 5.9          | 5.4-6.1 | 6.8     | 6.5-6.9 | 6.0-6.1       | ND      | 6.5-6.9 | 5.4-6.8 |
| Hemoglobin (g/L)                   | 120-125      | 110-123 | 136-141 | 137-142 | 129-130       | ND      | 134-145 | 110-134 |
| Hematocrit (L/L)                   | 0.4          | 0.3-0.4 | 0.4     | 0.4     | 0.4           | ND      | 0.4     | 0.4     |
| MCV (fl)                           | 60-63        | 62      | 61-63   | 62-63   | 61-64         | ND      | 61-63   | 63-66   |
| MCH (pg)                           | 20-21        | 20-21   | 20-21   | 21      | 21-22         | ND      | 20-21   | 20-21   |
| MCH Concentration (g/L)            | 336-339      | 329-333 | 328-332 | 327-335 | 334-341       | ND      | 329-334 | 319-327 |
| RDW (fL)                           | 14-15        | 14-15   | 13-15   | 15      | 14            | ND      | 14-16   | 14-15   |
| Platelet Count ( $\times 10^3/L$ ) | 0.9-1.0      | 1.0-1.1 | 1.4-1.7 | 1.3-1.5 | 0.6-1.0       | ND      | 1.0-1.6 | 0.5-1.6 |
| Mean Platelet Volume (fL)          | 6            | 5-6     | 6-7     | 6-7     | 6             | ND      | 6-7     | 6-9     |

ND – not determined, as all blood samples in this group were clotted  
RBC-Red Blood Cell; MCV – Mean Corpuscle Volume; MCH – Mean Corpuscle Hemoglobin; RDW – RBC Distribution Width

Table 4: Differential blood analysis from rats treated with control and fucoidan-loaded films following the caecal-sidewall surgical procedure

| Parameter   | Control Film |       |       |       | Fucoidan Film |       |       |       |
|-------------|--------------|-------|-------|-------|---------------|-------|-------|-------|
|             | Day 1        | Day 2 | Day 3 | Day 4 | Day 1         | Day 2 | Day 3 | Day 4 |
| Neutrophils | 8-12         | 7-16  | 4-11  | 5-7   | 17-23         | 7-8   | 3-9   | 6-16  |
| Lymphocytes | 74-89        | 81-91 | 82-91 | 89-92 | 71-78         | 86-90 | 89-94 | 80-91 |
| Monocytes   | 2-4          | 1-3   | 4-7   | 1-3   | 3-5           | 2-6   | 2-4   | 3-4   |
| Eosinophils | 1            | 0     | 0     | 1     | 0-1           | 0     | 0     | 0-1   |
| Bands       | 0            | 0     | 0     | 0     | 0-1           | 0     | 0     | 0-1   |

## CONCLUSIONS

Fucoidan film was safely administered during cecal-sidewall surgery in rats. Fucoidan film resulted in an approximately 90% reduction in adhesion score and an increase from zero to greater than 50% of animals being adhesion free. Fucoidan film is a promising candidate for further investigation in laparotomy procedures.

Table 1: Effect of locally administered sodium hyaluronate (HA) films loaded with various agents compared with untreated (no film) and unloaded HA films (control films) on adhesion formation and toxicity parameters following caecal-sidewall surgery in rats. Values were determined at time of sacrifice 7 days post-surgery and are reported as the mean  $\pm$  standard deviation.

| Treatment      | Number of animals | Adhesion Value                 | Animals Without Adhesions (%) | Incision Thickness (mm)      | WBC ( $\times 10^3$ ) |                            | Change in Initial Weight (%)  |
|----------------|-------------------|--------------------------------|-------------------------------|------------------------------|-----------------------|----------------------------|-------------------------------|
|                |                   |                                |                               |                              | Day 0                 | Day 7                      |                               |
| Untreated      | 17                | 10.2 $\pm$ 1.6                 | 0                             | 2.2 $\pm$ 0.3                | 1.2 $\pm$ 0.4         | 1.2 $\pm$ 0.5              | 0.7 $\pm$ 3.8                 |
| Control film   | 8                 | 5.8 $\pm$ 1.6 <sup>a</sup>     | 0                             | 2.2 $\pm$ 0.2                | 1.2 $\pm$ 0.2         | 1.2 $\pm$ 0.4              | 3.6 $\pm$ 2.8                 |
| Rapamycin      | 8                 | 6.6 $\pm$ 1.0 <sup>a</sup>     | 0                             | 1.6 $\pm$ 0.2 <sup>a,b</sup> | 1.3 $\pm$ 0.4         | 1.1 $\pm$ 0.4              | 1.0 $\pm$ 2.9                 |
| Celecoxib      | 8                 | 10.4 $\pm$ 2.0 <sup>b</sup>    | 0                             | 1.7 $\pm$ 0.3 <sup>a,b</sup> | 1.1 $\pm$ 0.2         | 1.0 $\pm$ 0.2              | 2.6 $\pm$ 3.2                 |
| Infliximab     | 8                 | 8.6 $\pm$ 2.7 <sup>a</sup>     | 0                             | 1.7 $\pm$ 0.4                | 1.4 $\pm$ 0.4         | 1.2 $\pm$ 0.3              | 3.3 $\pm$ 5.1                 |
| Docetaxel      | 4                 | 3.5 $\pm$ 1.3 <sup>a</sup>     | 25                            | 1.6 $\pm$ 0.1                | 0.8 $\pm$ 0.2         | 1.1 $\pm$ 0.5              | -7.0 $\pm$ 6.9 <sup>a,b</sup> |
| Cisplatin      | 4                 | 2.3 $\pm$ 2.1 <sup>a,b</sup>   | 25                            | 2.2 $\pm$ 0.2                | 1.2 $\pm$ 0.3         | 1.3 $\pm$ 0.3              | -4.8 $\pm$ 5.9 <sup>a,b</sup> |
| TIMP-2         | 4                 | 2.8 $\pm$ 1.3 <sup>a,b</sup>   | 0                             | 2.1 $\pm$ 0.1                | 1.2 $\pm$ 0.3         | 1.1 $\pm$ 0.3              | 0.9 $\pm$ 4.7                 |
| Heparin        | 4                 | 6.8 $\pm$ 1.5 <sup>a</sup>     | 0                             | 2.3 $\pm$ 0.2                | 1.4 $\pm$ 0.4         | 1.3 $\pm$ 0.4              | 2.8 $\pm$ 1.6                 |
| Indomethacin   | 4                 | 4.5 $\pm$ 1.0 <sup>a</sup>     | 0                             | 2.0 $\pm$ 0.2                | 1.6 $\pm$ 0.5         | 1.2 $\pm$ 0.2              | 1.1 $\pm$ 2.5                 |
| Dexamethasone  | 4                 | 4.3 $\pm$ 3.5 <sup>a</sup>     | 25                            | 1.7 $\pm$ 0.2 <sup>a,b</sup> | 0.9 $\pm$ 0.1         | 0.9 $\pm$ 0.4              | -5.9 $\pm$ 4.9 <sup>a,b</sup> |
| Pentoxifylline | 4                 | 6.3 $\pm$ 2.4 <sup>a</sup>     | 0                             | 2.2 $\pm$ 0.2                | 1.2 $\pm$ 0.2         | 1.1 $\pm$ 0.4              | 1.1 $\pm$ 3.4                 |
| Simvastatin    | 4                 | 4.8 $\pm$ 1.5 <sup>a</sup>     | 0                             | 2.2 $\pm$ 0.2                | 1.5 $\pm$ 0.4         | 1.4 $\pm$ 0.5              | 3.9 $\pm$ 2.4                 |
| Fucoidan       | 12                | 0.6 $\pm$ 2.3 <sup>a,b,c</sup> | 58                            | 2.3 $\pm$ 0.2                | 1.2 $\pm$ 0.4         | 1.1 $\pm$ 0.6              | 2.7 $\pm$ 3.6                 |
| HPC            | 4                 | 5.0 $\pm$ 1.2 <sup>a</sup>     | 0                             | 2.3 $\pm$ 0.2                | 0.8 $\pm$ 0.2         | 1.7 $\pm$ 0.1 <sup>b</sup> | 2.3 $\pm$ 3.1                 |

TIMP-2 = tissue inhibitor of matrix metalloproteinase-2; HPC = hydroxypropylcellulose.  
<sup>a</sup> Statistically significant compared with untreated group; <sup>b</sup> Statistically significant compared with control film group; <sup>c</sup> Statistically significant compared with all other groups

D. As presented in Table 6, blood chemistry parameter values showed no difference between control film and fucoidan film treated groups.

Table 5: Effect of 33% w/w fucoidan film compared with control film on blood chemistry parameter values following caecal-sidewall surgery in rats. Data represent the range of 3 rats per group.

| Parameter                                      | Control Film |           | Fucoidan Film |           |
|--|--------------|-----------|---------------|-----------|
|  | Day 3        | Day 4     | Day 3         | Day 4     |
| Glucose (nmol/L)                               | 9.3-14.7     | 8.7-15.3  | 10.8-12.8     | 10.0-13.8 |
| Blood Urea Nitrogen (nmol/L)                   | 5.7-6.7      | 6.0-6.8   | 4.4-6.2       | 6.3-8.7   |
| Creatinine ( $\mu$ mol/L)                      | 41-58        | 55-69     | 33-51         | 50-67     |
| Sodium (nmol/L)                                | 146-147      | 146-148   | 146-147       | 145-147   |
| Potassium (nmol/L)                             | 5.1-5.9      | 5.2-5.9   | 5.5-5.8       | 5.9-7.1   |
| Calcium (nmol/L)                               | 2.6-2.7      | 2.5-2.6   | 2.5-3.0       | 2.6       |
| Phosphorus (nmol/L)                            | 3.0-3.1      | 3.5-3.8   | 2.7-3.2       | 3.6-4.4   |
| Total protein (g/L)                            | 53-59        | 54-56     | 53-55         | 53-56     |
| Albumin (g/L)                                  | 29-30        | 30-31     | 28-30         | 31        |
| Globulin (g/L)                                 | 24-29        | 24-25     | 23-26         | 22-25     |
| Albumin/Globulin Ratio                         | 1.0-1.2      | 1.2-1.3   | 1.1-1.3       | 1.2-1.4   |
| Total Bilirubin ( $\mu$ mol/L)                 | 0-2          | 2         | 1-3           | 3         |
| Alkaline Phosphatase (IU/L)                    | 140-176      | 132-193   | 118-162       | 63-66     |
| Serum Glutamic Oxaloacetic Transaminase (IU/L) | 173-188      | 147-202   | 177-224       | 192-233   |
| Gamma Glutamyl Transpeptidase (IU/L)           | 0-3          | 0-6       | 0-2           | 0-3       |
| Chloride (mmol/L)                              | 106-107      | 105-106   | 106-109       | 107-108   |
| Carbon Dioxide (mmol/L)                        | 30-33        | 32        | 30-33         | 29-31     |
| Osmolarity (mmol/kg)                           | 299-302      | 300-304   | 300-301       | 301-304   |
| Anion Gap (mEq/L)                              | 12-17        | 14-16     | 12-14         | 14-17     |
| Creatine Phosphokinase (IU/L)                  | 949-1237     | 1080-1329 | 398-1446      | 1343-1722 |
| Serum Glutamic Pyruvic Transaminase (IU/L)     | 43-55        | 47-50     | 40-49         | 44-58     |

E. As shown in the urinalysis in Table 7, the pH of fucoidan film treated animals was significantly lower compared with the control film group. No other differences in urinalysis parameter values were noted between groups.

Table 6: Effect of 33% w/w fucoidan film on urinalysis parameter values following caecal-sidewall surgery in rats. Data represent the range of 3 rats per group.

| Parameter        | Control Film           |                        |         |         | Fucoidan Film          |                        |                        |                        |
|------------------|------------------------|------------------------|---------|---------|------------------------|------------------------|------------------------|------------------------|
|                  | Day 1                  | Day 2                  | Day 3   | Day 4   | Day 1                  | Day 2                  | Day 3                  | Day 4                  |
| pH               | 7.0-7.5                | 6.0-7.0                | 6.0-6.5 | 6.0-6.  | 5.0-6.0                | 5.0-7.0                | 6.0-7.0                | 5.0-8.0                |
| Specific gravity | 1.0-1.1                | 1.0-1.1                | 1.0-1.1 | 1.0-1.1 | 1.0                    | 1.1                    | 1.0-1.1                | 1.0-1.1                |
| Protein          | 1-5                    | 0-1                    | 4-7     | 4-7     | 0-1                    | 0                      | 0-1                    | 0                      |
| Glucose          | Neg                    | Neg                    | Neg     | Neg     | Neg                    | Neg                    | Neg                    | Neg                    |
| Ketone           | 0.5                    | 0.5                    | 0-0.5   | 0-0.5   | 0.5                    | 0.5                    | 0.5-1.5                | 0-1.5                  |
| Blood            | 1+2+                   | 1+4+                   | 0+3+    | 0+3+    | 3+4+                   | 1+                     | 0-4+                   | 0                      |
| Bilirubin        | Neg                    | Neg                    | Neg     | Neg     | Neg                    | Neg                    | Neg                    | Neg                    |
| Urobilinogen     | Norm                   | Norm                   | Norm    | Norm    | Norm                   | Norm                   | Norm                   | Norm                   |
| WBC              | 0-6                    | 0-6                    | Neg     | Neg     | 0-3                    | 0-6                    | 0-3                    | 0-3                    |
| RBC              | 0-3                    | 0-10                   | 0-3     | 0-3     | 0-6                    | 0-3                    | Neg                    | Neg                    |
| Epithelial cells | Few                    | Few                    | Neg-few | Neg-few | Few                    | Few                    | Neg-few                | Few                    |
| Hyaline cast     | Neg                    | Neg                    | Neg     | Neg     | Neg                    | Neg                    | Neg                    | Neg                    |
| Granulocyte cast | Neg                    | Neg                    | Neg     | Neg     | Neg                    | Neg                    | Neg                    | Neg                    |
| RBC cast         | Neg                    | Neg                    | Neg     | Neg     | Neg                    | Neg                    | Neg                    | Neg                    |
| Bacteria         | Neg                    | Neg                    | Neg     | Neg     | Neg                    | Neg                    | Neg                    | Neg                    |
| Mucus            | Neg                    | Neg                    | Neg     | Neg     | Neg                    | Neg                    | Neg                    | Neg                    |
| Crystals         | Triple PO <sup>+</sup> | Triple PO <sup>+</sup> | Neg     | Neg     | Triple PO <sup>+</sup> | Triple PO <sup>+</sup> | Triple PO <sup>+</sup> | Triple PO <sup>+</sup> |