

Anti-adhesive effects of the newly developed two-layered gelatin sheet in dogs

Abstract

Study question: Does the two-layered gelatin sheet which we newly developed prevent the cauterized uterus adhesion more effectively than the conventional anti-adhesive materials?

Summary answer: The two-layered gelatin sheet had significantly superior anti-adhesive effects to the conventional anti-adhesive materials.

What is known already: Hyaluronic acid carboxymethyl cellulose membrane (HA/CMC) is frequently used as a conventional anti-adhesive material clinically but the anti-adhesive effect of HA/CMC is controversial, concretely speaking it has reported to decrease severity of adhesion but not incidence in human and to have no effects to prevent pelvic adhesion in woman. Oxidized regenerated cellulose (TC7) is also a conventional material, however TC7 is only available to the non-bleeding site and provoke a large leucocyte response, which result in inducing adhesion.

Study design, size, duration: We have developed the two-layered gelatin sheet composed of gelatin film and gelatin sponge. Human fibroblasts and mesothelial cells were cultured on the materials for a week and the cell growth was evaluated. The dogs were randomly assigned to 4 groups: 1) control group, 2) two-layered gelatin sheet group, 3) HA/CMC group, and 4) TC7 group. The macroscopic and microscopic findings were evaluated on 3 and 6 weeks postoperatively.

Patients/materials, settings, methods: The viable cell number in each well was counted with the ATP assay. One side of the uterus horns was cauterized 40mm long circumferentially with electric scalpel. Adhesions were scored with a grading scale macroscopically. The cauterized site of the uterus were stained with hematoxylin-eosin (HE) and investigated histologically.

Main results and the role of chance: Cell growth of human fibroblasts and mesothelial cells were proliferated significantly on the two-layered gelatin sheet compared with the HA/CMC and TC7. The score in the two-layered gelatin sheet group was significantly lower than those in the HA/CMC and TC7 groups macroscopically. The degree of lymphocyte infiltration in the two-layered gelatin sheet group was sparse compared with those in the HA/CMC and TC7 group within 6 weeks. A single-cell layer of matured mesothelium was formed in the two-layered gelatin sheet group, however peritoneal regeneration in the HA/CMC and TC7 groups were incomplete and delayed. Inflammation around the each anti-adhesive material was the weakest in the two-layered gelatin sheet group. The anti-adhesive effects of two-layered gelatin sheet is superior to the conventional materials in the canine cauterized uterus model. Early regeneration of the peritoneum, weak inflammation, and longtime remain of the material contributes these results. The two-layered gelatin sheet is considered to be a useful anti-adhesive material in deeply injured and hemorrhagic sites.

Limitations, reasons for caution: The results are not easy to apply to human, because the structure of canine uteri are different from those of human. (21words<50)

Wider implication of the findings: The two-layered gelatin sheet is easier to use than the HA/CMC and TC7 in deeply injured and hemorrhagic sites or organ, because the sheet is able to be bent but not brittle.

Trial registration number: N/A

Keywords: anti-adhesive material, two-layered gelatin sheet.

Table 1. Adhesion Score

Category and Description	Score
Extent of site involvement	
None	0
≤25%	1
≤50%	2
≤75%	3
≤100%	4
Severity	
No adhesion	0
Adhesion falls apart	1
Adhesion lysed with traction	2
Adhesion required ≤50% sharp dissection	3
Adhesion required >50% sharp dissection	4

The degree of adhesion was scored by criteria of 0-4 for the extent or severity of the adhesion

Table 2.
 Histological findings of the site around materials

WEEKS	Gelatin sheet		Seprafilm®		INTERCEED®	
	3w	6w	3w	6w	3w	6w
EPITHELIUM REGENERATION	Yes	Yes	No	Yes	Yes	Yes
LAYER FORMATION OF MESOTHELIUM	Matured Single layer (Fig. 2a, 3a)		No	Immature	Poorly matured layer (Fig. 2c)	Matured Single layer (Fig.3c)
INFLAMMATION	Mild	Improved	Abundant macrophage Rich granulation		Abundant macrophage Rich granulation	
REMAINS OF MATERIAL	Fully remained	Little remained	Almost Ingested	Ingested	Almost Ingested	Ingested

Figure 1a

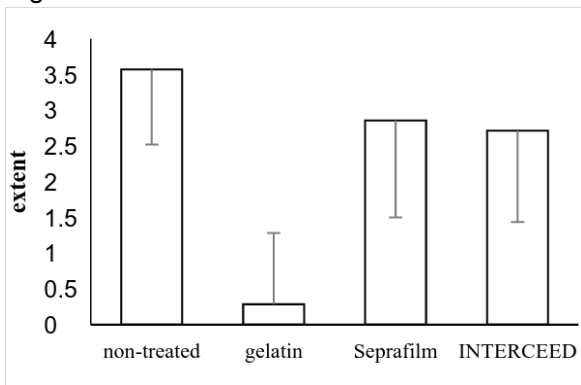


Figure 1b

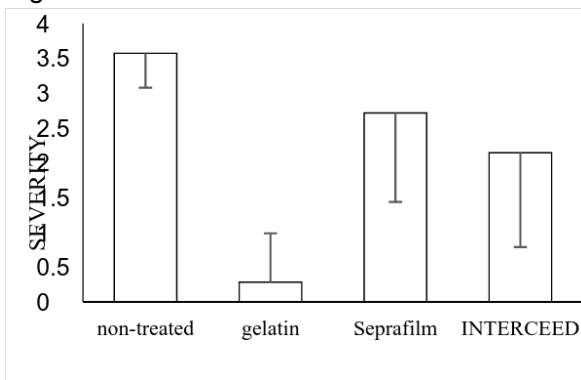


Figure 2a

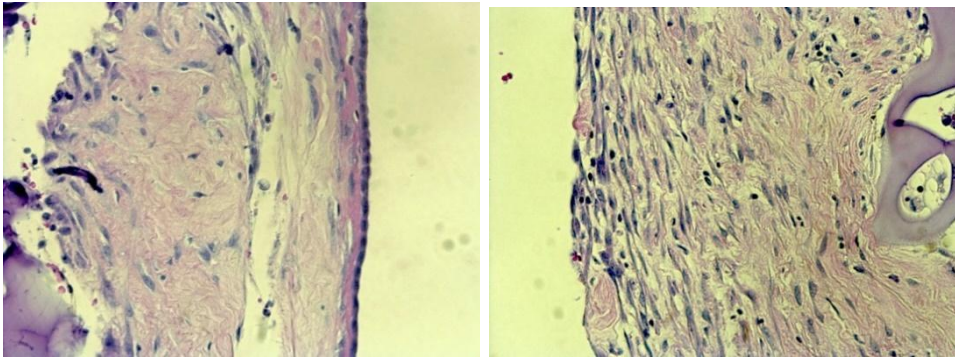


Figure 2b

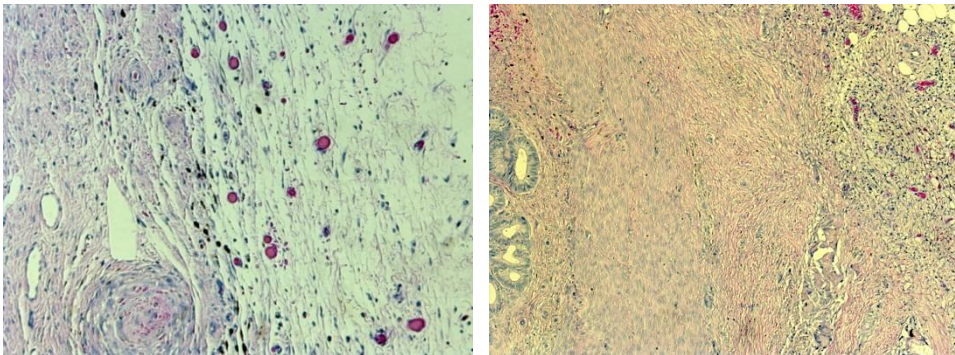


Figure 2c

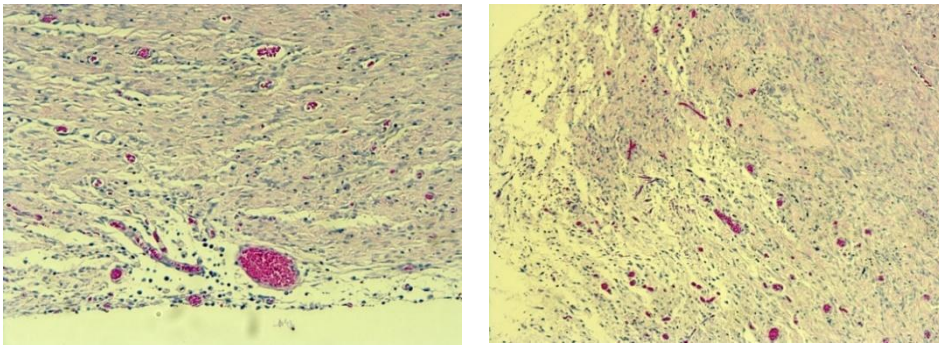


Figure 3a

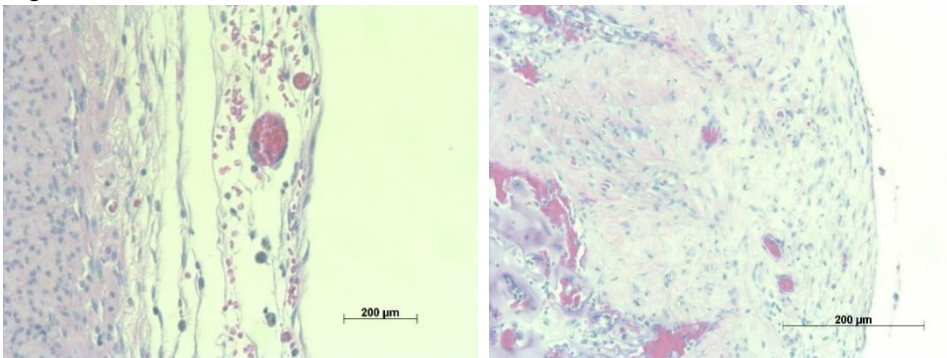


Figure 3b

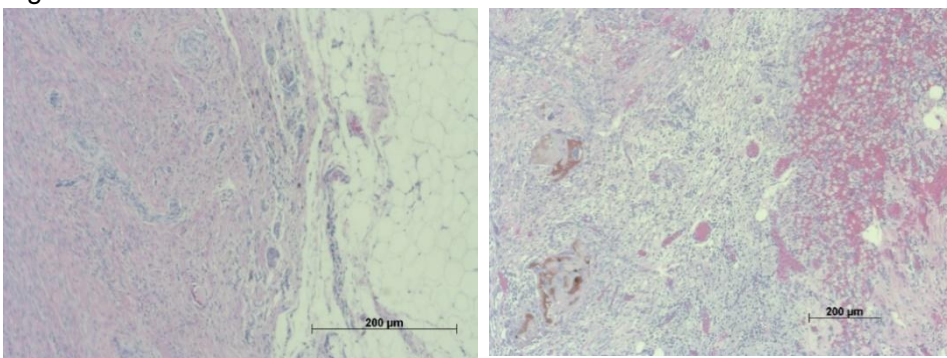


Figure 3c

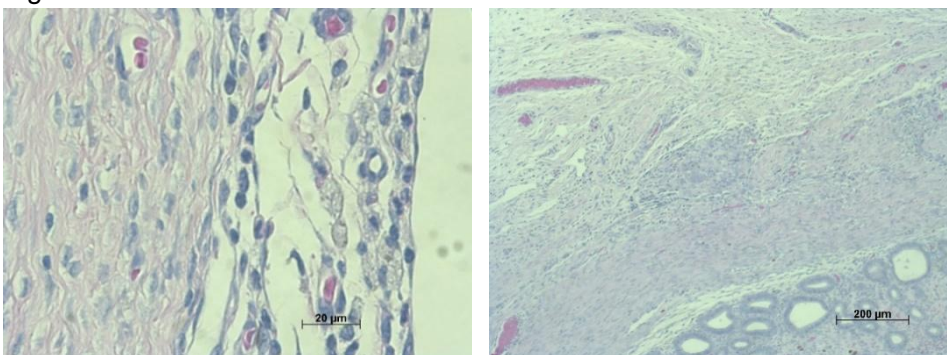


Figure 4a

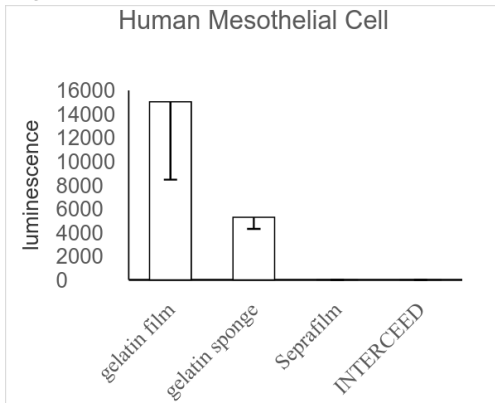


Figure 4b

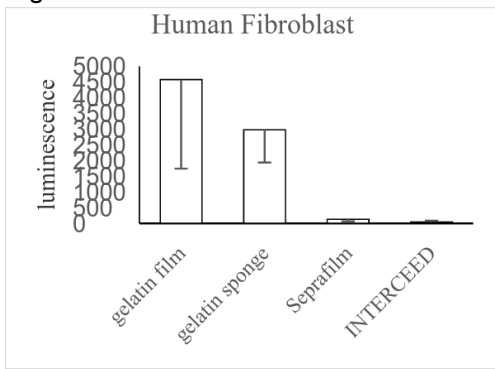


Figure 4c

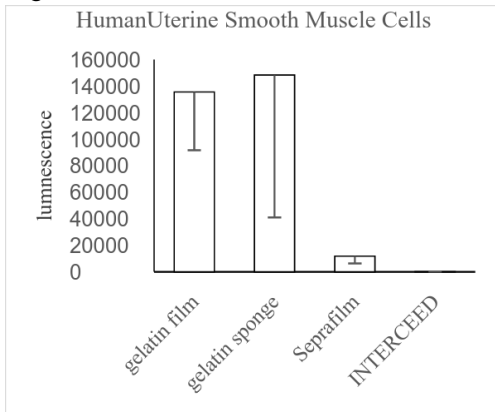


Figure legends

Figure 1. The adhesion scores of the anti-adhesive agents. 1a: the extent of adhesion. Two-layered gelatin sheet showed significant ($p < 0.05$) less score than non-treated group. 1b: the severity of adhesion. Two-layered gelatin sheet showed significant ($p < 0.05$) less score than non-treated group. Gelatin sheet showed no significant

($p < 0.05$) differences compared to Seprafilm® or INTERCEED®.

Figure 2. Histological findings 3 weeks after surgery. 2a: two-layered gelatin sheet. 2b: Seprafilm®. 2c: INTERCEED®.

Figure 3. Histological findings 6 weeks after surgery. 3a: two-layered gelatin sheet. 3b: Seprafilm®. 3c: INTERCEED®.

Figure 4. Cell growth on the materials (1 week). 4a: human mesothelial cells. 4b: humane fibroblasts. 4c: human uterine smooth muscle cells. Gelatin film and sponge groups showed significantly ($p < 0.01$) richer cell growth than Seprafilm or INTECEED group.

Cover letter

T.M. D'Hooghe, Leuven
Editor-in-Chief
Gynecologic and Obstetric Investigation

Dear Dr. T.M. D'Hooghe, Leuven:

We would like to ask you to consider our manuscript entitled “**Anti-adhesive Effects of the Newly Developed Two-Layered Gelatin Sheet in Dogs**” for publication in *Gynecologic and Obstetric Investigation* as an Original Article.

In this study, anti-adhesive effects of newly developed two-layered gelatin sheet were evaluated compared with conventional agents. The effects of two-layered gelatin sheet is superior to the conventional agents in cauterized uterus model. Early regeneration of the peritoneum, weak inflammation, and longtime remain of the material contributes the results.

No portion of the paper has been previously published, and the manuscript is not under consideration for publication by any other journal. The manuscript was checked by a native English speaker. The animal experiments performed in this study were approved by the Nara Medical University Experimentation Committee and the Doshisha University Animal Experimentation Committee. All animal care, housing, and surgical and anesthetic procedures were performed in accordance with the animal care guidelines of the Committee for Animal Research of Nara Medical University.

This work was supported, in part, by a grant from the Science and Engineering Institute of Doshisha University. No author has any other financial or personal relationship with people or organizations that could potentially and inappropriately influence our work and conclusions.

Thank you for considering this paper for publication in *Gynecologic and Obstetric Investigation*, and I await your response at your earliest convenience.

Sincerely,

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