The Effects of Thermally Cross-Linked Gelatin Film on Intraperitoneal Dissemination of Cancer Cells
-An In Vitro Study Using Human Gastrointestinal Cancer Cell Lines-

Hiroe MIYAMOTO*, Hiroyuki TSUJIMOTO**, Tsunehito HORII*, Junki IKEDA***, Taichi ORIKASA***,
Hideki TAKAMORI**, Hiroyuki TORII***,****, Yuki OZAMOTO****, Shinichiro MORITA****,******,
Mamoru URABE**,***** and Akeo HAGIWARA**

(Received January 15, 2013)

To prevent adhesion formation after abdominal surgery, we newly developed a thermally cross-linked gelatin film and previously reported its superior anti-adhesive effects with excellent peritoneal regeneration. However, since the gelatin film is made from a degenerated product of collagen which is the most abundant extracellular matrix protein in mammals and has been used as a scaffold in the field of regeneration medicine, it may act as a scaffold convenient for cancer cell growth, thereby accelerating peritoneal dissemination when used in surgery for abdominal cancers.

To clarify this issue, in this study, we examined effects of the gelatin film on in vitro cancer cell growth, using the human colon cancer cell line HT29 and the human gastric cancer cell lines MKN74 and KATO-III. HT29 and MKN74 cells exhibit an adherent growth mode and Kato-III cells exhibit a semi-floating growth mode in conventional culturing. The cancer cells were cultured on the gelatin film or without the film (control), and each viable cell number was counted at 1, 3, 5 and 7 days after seeding.

The growth of the HT29 and MKN74 cells was significantly less than that observed in the control group, although both cells grew on the gelatin film with time. Compared with the other two cell lines, the KATO-III cells seemed to grow on the gelatin films well. However, the cell growth was also less than that observed in the control group. The results of this in vitro study suggest that gelatin film may not act as a scaffold convenient for cancer cell growth or accelerate peritoneal dissemination, although further in vivo studies are needed.

Key words: gelatin film, anti-adhesion, cancer cells, peritoneal dissemination, scaffold

1. Introduction

Post-operative adhesion is frequently observed during the process of wound healing in patients undergoing abdominal and gynecologic surgery1). Intra-abdominal adhesion often causes several complications2,3 such as intestinal obstruction4), female infertility5) and chronic abdominal pain. To dissolve the problem of adhesion, various kinds of anti-adhesive materials have been developed experimentally and

* Undergraduate Sophomore, Department of Medical Life System, Doshisha University, Kyoto
Telephone:+81-774-65-6878, E-mail: bml2049@mail4.doshisha.ac.jp
** Department of Medical Life System, Doshisha University, Kyoto
*** Graduate Student, Department of Medical Life System, Doshisha University, Kyoto
**** Research and Development Department, Gunze Ltd., Ayabe-shi, Kyoto
***** Kusatsu General Hospital, Shiga
****** Bio-Medical Material Research Center, Doshisha University, Kyoto
Corresponding Author: Akeo HAGIWARA, Telephone:+81-774-65-6878, E-mail: ahagiwar@mail.doshisha.ac.jp

(16)
Effects of Gelatin Film on Dissemination

Clinically so far.

Presently, cellulose film, which is composed of a combination of sodium hyaluronate and carboxymethyl-cellulose, is commonly used in clinical practice as a bioabsorbable anti-adhesive material. Cellulose film has been reported to turn into a gel when placed on an injured site and to remain on the site for up to seven days, acting as a mechanical barrier that separates the injured site from the adjacent tissues. Indeed, some reports indicated that cellulose film reduced adhesion formation in open abdominal surgery.

However, cellulose film has some disadvantages. One is the difficulty of handling the film during surgery because it is fragile and tears easily. The other is that the use of cellulose film is contraindicated to wrap anastomoses directly because it induced a high incidence of anastomotic leakage.

To overcome these problems, we developed a thermally cross-linked gelatin film as a new anti-adhesive material. Previously, we reported that this gelatin film prevented adhesion formation more effectively than cellulose film with excellent peritoneal regeneration and could also be used on anastomotic sites safely in canine models.

As known well, gelatin is a degenerated product of collagen which is the most abundant extracellular matrix protein in mammals. Therefore, gelatin film may have beneficial effects for repairing injured tissues as well as anti-adhesive effect, similar to collagen which has been used as a scaffold in the field of regenerative medicine. However, when gelatin film is used in patients with cancers of the abdominal organs, it may also act as a scaffold convenient for cancer cell growth, thereby accelerating peritoneal dissemination.

To clarify this issue, in this study, we examined the effects of gelatin film on in vitro cancer cell growth using human gastrointestinal cancer cell lines, compared with cellulose film.

2. Materials and Methods

2.1 The films

Medical grade alkali-treated gelatin (Medigelatin®) extracted from porcine skin with an isoelectric point of 5 was purchased from Nippi Co. Ltd. (Tokyo, Japan). To prepare the gelatin film, the gelatin was dissolved in distilled water at a concentration of 4.5%. The gelatin solution was cast on plastic plates and allowed to air-dry to obtain films of approximately 30 µm in thickness. Then, the gelatin film was cross-linked thermally using a vacuum oven (4VO-250N, As One, Osaka, Japan) at 140°C for three hours. Finally, the film was sterilized with ethylene-oxide gas (0.43g/L at 40°C for four hours) for further in vitro examinations.

Cellulose film (Seprafilm®, Genzyme Ltd. Cambridge, MA, USA) with a thickness of approximately 50µm was purchased from Kaken Pharmaceutical Co. Ltd. (Tokyo, Japan).

2.2 The cell lines

The human colon cancer cell line HT29 was purchased from the American Type Culture Collection (Rockville, MD, USA). The human gastric cancer cell lines MKN74 and KATO-III were obtained from the Human Science Research Resources Bank (Osaka, Japan). Based on previous reports, HT29 and MKN74 cells exhibit an adherent growth mode and Kato-III exhibit a semi-floating growth mode in conventional culturing. HT29 and MKN74 cells were maintained in Dulbecco’s modified Eagle medium (D-MEM, Wako Pure Chemical Industries, Ltd. Osaka, Japan) containing 10% fetal bovine serum. KATO-III cells were cultured in RPMI 1640 medium (Wako Pure Chemical Industries, Ltd. Osaka, Japan) containing 10% fetal bovine serum. The cells were cultivated in an incubator at 37°C with 5% CO2 under humid conditions.

2.3 Cell growth assay

For the cell-culture, we used 24-well culture plates without any coating (Becton, Dickinson and Company Ltd. Franklin Lakes, NJ, USA), which had wells of 15mm in diameter. The gelatin film and the
cellulose film were cut into a circle measuring 15mm in diameter, so that the circles completely covered the bottoms of the wells. The wells in the control group were not covered by any films. The cultured cancer cells were harvested in the form of a single cell-suspension. The cell suspension containing 2.0×10^3 cells/well was poured into the wells with each film or without film. These cells were cultivated up to seven days.

One, 3, 5 and 7 days after seeding, the viable cell number in each well was counted with the ATP assay using an ATPLite Kit (Perkin Elmer, Inc. Waltham, MA, USA). For each time point, three wells for each experimental group were examined.

2.4 Statistical analysis

The cell growth was analyzed using a one-way analysis of variance (ANOVA) and the Tukey test as a post hoc-test. A P value of <0.05 was determined to be statistically significant.

3. Results

The results of the growth test for the HT29 cells are shown in Fig.1. The HT29 cells grew on the gelatin film as well as in the control wells with time. However, the growth was significantly lower than that in the control group on day 3 (P<0.01), 5 (P<0.01), and 7 (P<0.001). In contrast, the HT29 cells on the cellulose film did not grow and rather the number of cancer cells diminished with time.

The results of the growth test for the MKN74 are shown in Fig.2. Similar to the HT29 cells, the MKN74 cells grew on the gelatin film with time, and the growth was significantly lower than that observed in the control group on days 1 (P<0.05), 3 (P<0.001), 5 (P<0.001) and 7 (P<0.001). In the cellulose group, the number of MKN74 cells also diminished.

The results of the growth test for the KATO-III cells are shown in Fig.3. Compared with the other cancer cells (HT29 and MKN74), the KATO-III cells seemed to grow well on the gelatin film. However, the growth was significantly lower than that observed in the control group on days 1 (P<0.05), 5 (P<0.01), and 7 (P<0.05). In the cellulose group, the number of Kato-III cells diminished similar to the other cancer cells.

Fig.1. Growth of HT29 cells. ■ The control group, The gelatin film group, □ The cellulose film group. *: P<0.05. **: P<0.01. +: P<0.001.

Fig.2. Growth of MKN74 cells. ■ The control group, ■ The gelatin film group, □ The cellulose film group. *: P<0.05. **: P<0.01. +: P<0.001.
Fig. 3. Growth of KATO-III cells. ■ The control group, □ The gelatin film group, △ The cellulose film group.
*: P<0.05. **: P<0.01. +: P<0.001.

4. Discussion

In this experiment, the human colon cancer HT29 cells and the human gastric cancer MKN74 cells grew on the thermally cross-linked gelatin film with time. However, the growth was significantly less than that observed in the control group, which did not use any film. In addition, the human gastric cancer KATO-III cells also grew on the gelatin film at a rate that was significantly less than that observed in the control group. Based on these results, the gelatin film hardly acts as a scaffold convenient for cancer cell growth.

In a preliminary experiment, we confirmed that approximately 85% of the gelatin film dissolved in distilled water at 37°C after seven days (data not shown). Similarly, the gelatin film in our experiment was considered to dissolve gradually during the culture. HT29 and MKN74 cells exhibit an adherent growth mode in conventional culturing. Therefore, due to the dissolving of the gelatin film, these cells may not adhere constantly to the gelatin film as a scaffold and not grow well on the film due to so-called “adhesion inhibition”, compared with the control group.

In contrast, KATO-III cells exhibit a semi-floating growth mode in conventional culturing. In our experiments, although the growth of the KATO-III cells was less than that observed in the control group, it seemed to be better than that of the HT29 and MKN74 cells. The growth of KATO-III cells may not be suppressed only by “adhesion inhibition”, as compared with the HT29 and MKN74 cells.

Interestingly, the growth of all of the cancer cells on the cellulose film was remarkably suppressed, irrespective of their growth modes. This result indicates that the cellulose film, otherwise its degraded products, may impair the growth of these cancer cells more directly as well as by “adhesion inhibition”. In this scenario, the cellulose film may suppress peritoneal dissemination of cancers. However, previous experiments examining the effects of cellulose film on peritoneal dissemination using murine cancer models have reported no significant differences between no-treatment groups and groups treated with the cellulose film\(^{16, 17}\). Therefore, the results of this \textit{in vitro} experiment may not necessarily correspond to those of the \textit{in vivo} experiment.

In conclusion, based on the results of this \textit{in vitro} study, the thermally cross-linked gelatin film may not act as a scaffold convenient for cancer cell growth or accelerate peritoneal dissemination when used in surgery for abdominal cancers. However, further \textit{in vivo} examinations are needed to clarify this issue.

References


