J Mater Sci: Mater Med (2007) 18:475–482
DOI 10.1007/s10856-007-2007-z

Preparation and characterization of biodegradable anti-adhesive membrane for peritoneal wound healing

Si-Nae Park · Han Jeong Jang · Yu Suk Choi · Jae Min Cha · Seo Yeon Son · Seung Hun Han · Jung Hyun Kim · Woo Jung Lee · Hwal Suh

Received: 19 April 2005 / Accepted: 29 October 2005
© Springer Science + Business Media, LLC 2007

Abstract Postoperative adhesions remain a significant complication of abdominal surgery although the wide variety of physical barriers has been developed to reduce the incidence of adhesion. In this study, the bilayered composite membrane formed by the association of a methoxy poly (ethylene glycol)-poly (L-lactide-*co*-glycolide) (mPEG-PLGA) film and a crosslinked collagen-hyaluronic acid (Col-HA) membrane with fibronectin (FN) coating was prepared for promoting wound healing and providing tissue adhesion resistance simultaneously. *In vitro* adhesion test revealed that fibroblasts attached better on Col-HA membrane compared to those on mPEG-PLGA film, PLGA film or Interceed^{TM} (oxidized cellulose) while mPEG-PLGA film had the lowest cell adhesive property. In confocal microscopic observation, the actin filaments were significantly further polymerized when 50 or 100 μg/cm^3 fibronectin was incorporated on the COL-HA membranes. After 7-day culture, fibroblasts penetrated throughout the Col-HA-FN network and the cell density increased whereas very few cells were found attached on the surface of the mPEG-PLGA film. *In vivo* evaluation test showed that the composite membrane could remain during the critical period of peritoneal healing and did not provoke any inflammation or adverse tissue reaction.

S.-N. Park · H. Suh (✉)
BK 21 Project Team of Nanobiomaterials for the Cell-based Implants, Yonsei University, 134 Shinchon-dong, Seodaemun-ku, Seoul 120-749
e-mail: hwal@yumc.yonsei.ac.kr

Y. S. Choi · J. M. Cha · S.-N. Park · H. Suh
Department of Medical Engineering, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-ku, Seoul 120-752, Korea

H. J. Jang · W. J. Lee
Department of General Surgery, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-ku, Seoul 120-752, Korea

S. Y. Son · S. H. Han · J. H. Kim
Nanosphere Process and Technology Laboratory, Division of Chemical Engineering and Biotechnology, Yonsei University, 134 Shinchon-dong, Seodaemun-ku, Seoul 120-749, Korea

Introduction

Postoperative adhesion, which occurs after most abdominal surgery, is the development of abnormal attachments between tissues and organs in response to trauma [1]. Whether associated with general, gynecological, thoracic, or orthopedic surgery, postoperative adhesions can cause severe and life-threatening complications [2], including intestinal obstruction, infertility and pain.

To date, most efforts to prevent or reduce adhesion formation have met with little success. This includes the use of agents directed at modifying the adhesion-producing inflammatory cascade and those intended to provide a temporary physical barrier between injured tissue surfaces during the early stages of wound repair [3].

Although the concept of a barrier is simple, a barrier must meet a variety of requirements to merit wide acceptance [4]. It must be safe, noninflammatory and nonimmunogeneic, that is, not itself provoke inflammation, infection, fibrosis, or encapsulation. The barrier should be completely biodegradable, yet maintain an adequate residence time. It should be rapidly and easily applied, including by laparoscopy, if possible, and it should remain stationary without the need for sutures or staples. Currently, two kinds of barrier, oxidized regenerated cellulose (Interceed^{TM} (TC7), Ethicon, Inc., Somerville, NJ, USA) [5] and Seprafilm Bioresorbable Membrane (Genzyme, Corp., Cambridge, MA, USA) [6, 7] have been approved for use in the United States. Other

products, INTERGEL (Lifecore Biomedical, Chaska, MN, USA) [8] and ADEPT (ML Laboratories Ltd., Leicester, UK) are also available in other parts of the world.

As an implantable material for the repair of damaged tissues, collagen (Col), probably the most promising wound dressing material, has been proven effective in wound healing [9, 10]. Hyaluronic acid (HA) has been also widely used as the base material for artificial skin, as an injectable tissue expansion solution. These biodegradable extracellular matrix analogs can enhance the restoration of damaged peritoneal wall after surgery but may cause adhesion to the underlying viscera.

In our laboratory, the bi-layered composite membrane formed by the association of a methoxy poly (ethylene glycol)-poly (L-lactide-co-glycolide) (mPEG-PLGA) film and a crosslinked Col-HA membrane was prepared as a post-operative anti-adhesive membrane. The Col-HA membrane side of bilayered composite membrane was intended to face the damaged and bleeding area for promoting wound healing while the mPEG-PLGA side prevents neighboring tissues to adhere. *In vitro* cell adhesion test was conducted by the culture of human fibroblasts on the composite membrane. To further enhance the bioadhesive property of Col-HA membrane side, we coated fibronectin (FN) on the membrane at various concentrations, and examined the effect of FN on the polymerization of actin filaments of adhered fibroblasts. The degradation rate was also determined by the change in the membrane's mass and the molecular weight of the mPEG-PLGA. For the evaluation of *in vivo* tissue compatibility, the membrane was implanted into the sidewall defect of porcine abdomen.

Materials and methods

mPEG-PLGA block copolymer film

PLGA (Mw. 90,000–126,000) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). mPEG-PLGA block copolymer (Mw. 14000) was co-polymerized through the ring-opening polymerization. In brief, glycolide (Boehringer Ingelheim, Ingelheim, Germany) and D,L-lactide (Boehringer Ingelheim) were recrystallized from ethyl acetate and dried under vacuum before use. Lactide and glycolide, at a molar ratio of 75:25, were put in Pyrex® ampoule. mPEG (Mw. 550, Sigma-Aldrich, St. Louis, MO, USA) was added for preparing the mPEG-PLGA block copolymer. 0.05% (w/w) of stannous octoate (Sigma Chemical Co., St. Louis, MO, USA) was added to the solution as a catalyst. The ampoule was evacuated using a vacuum pump and sealed with a torch. The ampoule was heated in the oil bath at 130°C for 12 hrs. After the reaction was complete, the resulting polymers were purified by dissolving in methylene chloride and then precipitated in excess methanol. The obtained polymers were dried under vacuum.

For the preparation of polymer film, an 8% solution of mPEG-PLGA or PLGA, in chloroform, was cast on a glass plate and the solvent was evaporated in a vacuum oven for 2 days.

mPEG-PLGA/Col-HA-FN composite membranes

The porous Col-HA membrane was fabricated and crosslinked, as previously reported [11]. 1% of hyaluronic acid (HA) (sodium salt, Mw = 120,000-150,000, Hanwha group R&D Center, Daejeon, Korea) as an aqueous solution was added to a 1% type I atelocollagen (RBC I, Regenmed, Seoul, Korea) dispersion, forming a 20% (w/w) HA/Col solution and homogenized (T25 basic, IKA Works, Selangor, Malaysia) at 8000 rpm for 3 min at 4°C. The resulting slurry was poured into a well plate (diameter 10 mm), frozen at –70°C, and then lyophilized at –50°C. The fabricated porous Col-HA membranes obtained were immersed in 50 mM of 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma Chemical Co., St. Louis, MO, USA) solution (H_2O-ethanol = 5:95) for 24 h. The membranes obtained were washed in D.W using a sonicator, and then re-lyophilized at –50°C.

The Col-HA membranes coated with various concentrations of FN, were prepared by applying 1% FN solution over the Col-HA membranes at doses of 30, 50 or 100 μg/cm^3 and incubation at 37°C under humid condition for 5 hours.

For preparing the bi-layered composite membrane, chloroform was sprayed on the surface of mPEG-PLGA film using a glass sprayer, and then cross-linked Col-HA or Col-HA-FN membrane was loaded on the slightly dissolved surface of mPEG-PLGA film. Residual solvent was removed by evaporation in a vacuum oven for 2 days. To examine the integrity of bilayered membrane, a cross-section of the membrane was coated with an ultrathin layer (300 Å) of gold/Pt in an ion sputter (E1010, HITACHI, Tokyo, Japan) and observed by scanning electron microscopy (S-800, HITACHI, Tokyo, Japan).

Cell adhesion assay

Fetal human dermal fibroblasts were obtained from the department of plastic surgery, Yonsei Medical Center, Seoul, Korea, with permission from the Medical Material Control and Management Committee of Yonsei University. Outgrown cells were taken from primary culture, following established methods [12]. Cells were cultured in 175 cm^2 tissue culture flasks (NUNC, Roskilde, Denmark) using growth medium composed of DMEM (JBI Inc, Daejeoun, Korea), 1% penicillin/streptomycin/amphotericin-B, and 10% fetal

bovine serum (FBS, JBI Inc, Daejeoun, Korea). Cultures were incubated in a humidified incubator in an atmosphere of 5% CO_2 and 95% air. Confluent monolayers were propagated by trypsinization (0.25% trypsin, 0.02% EDTA solution) and replating at 1:2 dilution. For the experiments, fibroblasts were used in the fifth-seventh passages.

100 μl of cell suspension containing 2×10^5 cells in culture medium with or without FBS was placed onto the PEG-PLGA film, PLGA films, the collagen-HA membrane or Interceed^{TM}(10 mm in diameter). 1 ml of culture medium with or without FBS was added 1hour after seeding, and the cells were allowed to attach for 4 h.

The attached cells were then quantified by the 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) assay [13, 14]. 50 μl of MTT solution (5 mg/ml in 0.9% NaCl, filter-sterilized) and 200 μl of medium were added to each culture well. After incubation for 4 h, the MTT reaction medium was removed, and 300 μl of dimethylsulfoxide and 25 μl of Sorensesn's glycine buffer (0.1 M glycine, 0.1M NaCl adjusted to pH 10.5 with 1M NaCl) was added. Optical densities were determined by ELISA reader (Spectra Max 340, Molecular Device Inc., CA, USA) at a wavelength of 570 nm. For microscopic analysis, the cell attached membranes were washed with 0.1 M PBS and then fixed in Karnovsky's fixation solution (2% glutaraldehyde, 2% paraformaldehyde and 0.5% $CaCl_2$) for 6 h. After washed 3 times with 0.1 M PBS, samples were postfixed with 1% OsO_4 in PBS for 1 hr and then washed with 0.1 M PBS. The samples were dehydrated through a graded series of ethanol, critical-point dried, coated and then examined by SEM, as described above.

For examining cell proliferation on the composite membrane, 10^5 cells were seeded onto each side of the mPEG-PLGA/Col-HA composite membrane (10 mm in diameter) and harvested for 7 days. The cultured specimens were then fixed in 10% neutral-buffered formalin solution, embedded in paraffin wax, sectioned in 3 μm slices, stained with hematoxylin-eosin (H-E) for histological observation.

F-actin staining

We used a modification of the methods of Steven et al. [15] to visualize the polymerization of filamentous actin (F-actin) fibers within the adherent fibroblasts on mPEG-PLGA film, Col-HA membrane, Col-HA-FN membrane side and Interceed^{TM} 4-h after attachment. Adherent cells were fixed in 3.7% paraformaldehyde-phosphate buffered saline (PBS), containing 0.1% Triton X-100, for 10 min at room temperature (pH 7.2). The cells were then washed twice with PBS and subsequently permeabilized with 0.2% Triton X-100 in PBS for 10 min. To localize the F-actin, the cells were stained with fluorescein isothiocyanate (FITC)-labeled phalloidin (5 μg/ml) in blocking solution (1% BSA-0.1% Triton X-100 in PBS) in a dark room overnight at room temperature. The next day, the cells were extensively washed with PBS. Propidium Iodide (PI) solution was added for nuclear counterstaining.

The F-actin staining intensity within the cells was calculated using the MetaMorph software (Universal Imaging Corp., West Chester, PA, USA). The average pixel intensity (a digital expression of average fluorescence) was generated for at least five cytoplasmic regions of each cell. Regions containing cell margins or nuclear profiles were not included. For each experiment, the F-actin staining intensity of at least 10 cells from different fields was averaged for a single datum point. An increase in the F-actin staining intensity indicated an increase in actin reorganization [15]. Significance levels were determined by ANOVA, with P values of <0.05 were considered significant.

Degradation testing

The *in vitro* degradation rate was determined by the change in the sample's mass and the molecular weight of the mPEG-PLGA. The mPEG-PLGA/Col-HA composite membranes were immersed in 5 ml of phosphate buffered saline, containing 2% penicillin/streptomycin/amphotericin-B agent, and incubated at 37°C and 20 rpm. After 3, 7 and 14 days of incubation, the samples were freeze-dried and weighed. The molecular weight of mPEG-PLGA copolymer was analyzed by gel permeation chromatography.

Preliminary animal study

A young female Yorkshire pig (35 kg) was used in this study. The animal experimental procedure was managed in accordance with the Guidelines and Regulations for Use and Care of animals in Yonsei University. All the animals were fasted overnight and given free access to water. The animals were sedated by intramuscular injection of ketamine 10 mg/kg, xylazine 2 mg/kg, and atropine 0.05 mg/kg. They were anesthetized by injection of 6 mg/kg propofol intravenously and intubated. The animals were ventilated at a rate of 10 to 13 breaths per minute, and anesthesia maintenance was achieved with enflurane (1–2.5% in 100% oxygen; SA2/RA2 inhalation anesthetic machine with electric ventilator, Dräger Medical AG&Co. KGaA, Lübeck, Germany). The animals were kept in the supine position throughout the experiment. An incision line marked on the skin overlying the linea alba on the ventral midline and then the skin was retracted and undermined slightly to facilitate the suturing of the skin at the end of the procedure. With the muscle wall exposed, an incision was made along the linea alba though to the peritoneal cavity. The right abdominal wall was reflected, and a 3-cm^2 defect was created by removing the peritoneum and some associated muscle fibers. A piece of the composite membrane consisting of mPEG-PLGA film and 50 μg/cm^3 FN coated

Col-HA membrane was prepared to be 4 cm × 4 cm larger than all of the measured dimensions of the defect and thus to allow for a 1-cm overlap approximately. The membrane, with Col-HA-FN membrane side facing the peritoneum, was secured by suturing the eight points using a 6-0 polypropylene suture. At the left abdominal wall, the same procedure was performed, but no membrane was placed in the abdominal cavity. 7 days after the operation, the animal was sacrificed with intramuscular ketamine (10 mg/kg) and Rompun® (2 mg/kg, Bayer, Leverkusen, Germany), intravenous propofol (6 mg/kg), followed by potassium chloride (150 mg/kg). The abdominal wall was circumferentially incised to expose the repair site and photographs were taken. A transverse section of full-thickness abdominal wall through the center of the repair site, including adjacent abdominal wall, was excised, fixed in 10% formalin, embedded in paraffin wax, sectioned at 3 μm thickness, and stained with hematoxylin-eosin stain for histological examination.

Statistical analysis

Significance levels were determined by ANOVA. Multiple comparisons were carried out by the Tukey method. All statistical calculations were done on the SAS system for Windows (version 8.00, SAS Institute Inc., Cary, NC, USA). P values of <0.05 were considered significant.

Results and discussion

Fabrication of the composite membrane

Figure 1 illustrates the cross-sectional morphology of the composite membrane, which consists of mPEG-PLGA film and Col-HA membrane. The Col-HA membrane part was porous due to the water, which had been homogeneously dispersed in the Col-HA mixture prior to freezing [11] and the three-dimensional interconnected porosity was maintained after the association with mPEG-PLGA film. Figure 1(B) showed that the two layers of mPEG-PLGA/Col-HA composite membrane were tightly contacted without peeling.

In vitro adhesion assay

SEM observations revealed that the fibroblasts attached evenly and had well stretched pseudopodia on the Col-HA membrane whereas the majority of cells on the mPEG-PLGA film or Interceed™ matrix were localized and spherical in shape (Fig. 2).

The number of cells adhered on the Col-HA membrane, control PLGA film, mPEG-PLGA film and Interceed™ membrane were compared in the medium both with and without serum, as shown in Fig. 3. Four hours after seeding,

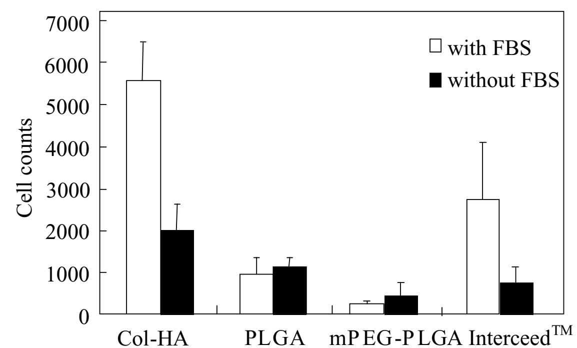

Fig. 3 Attachment of fetal human dermal fibroblasts on the various types of membrane 4 h after seeding: Cell adhesion on the Col-HA membrane, control PLGA film, mPEG-PLGA film and Interceed™ membrane were compared in the culture medium with or without fetal bovine serum.

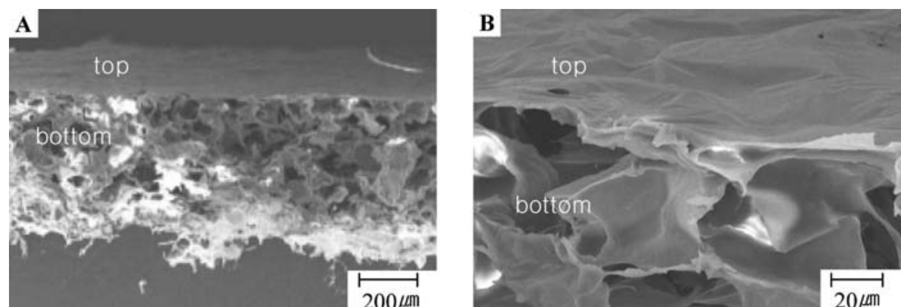

Fig. 1 Scanning electron microscopy of cross-section of the composite membrane consisting of mPEG-PLGA film and porous Col-HA layer. Top: mPEG-PLGA film layer, bottom: freeze-dried, crossliked Col-HA membrane. (A) (magnification ×50) , (B) (magnifaction ×500).

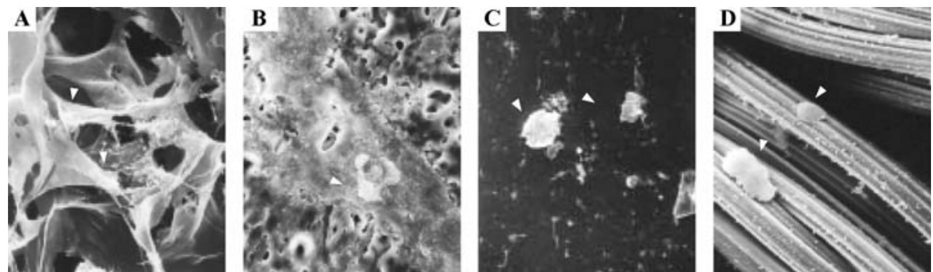

Fig. 2 Scanning electron microscopy observation of attached fibroblasts on the Col-HA matrix (A), PLGA film (B), mPEG-PLGA film (C) and Interceed™ (D) (magnification ×500).

greater number of fibroblasts attached to Col-HA matrix as compared with the cells on PLGA film, mPEG-PLGA film or Interceed™, while the mPEG-PLGA film had the lowest cell adhesive properties. Serum containing medium increased the numbers of cells attaching on the collagen-HA matrix and Interceed™. However, no significant effect of serum in the medium on the cell attachment to PLGA or mPEG-PLGA film was found. The anti-adhesive property of mPEG-PLGA can be explained by the interfacial free energy, where the decrease in the free energy due to end-grafted PEG chains diminishes the protein adsorption and cell adhesion to the surfaces [16]. Another reason for the hampered attachment of cells could be an alteration in the surface hydrophilicity caused by PEGylation [17].

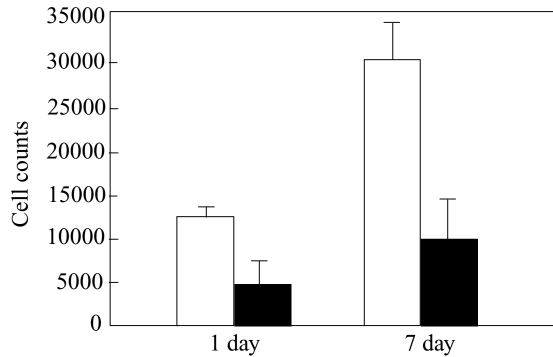

Fig. 4 Cell proliferation assessed by MTT testing performed 1 day and 7 day after culturing. The data reported for mPEG-PLGA films and Col-HA membranes are means ± SD for $n = 5$. ■: mPEG-PLGA film, □: Col-HA membranes.

To verify that cells were not only attached on the membranes, but also viable and had the capability to proliferate well, we examined the cell proliferation on each side of the PEG-PLGA film/Col-HA composite membrane by H&E staining and MTT assay. The number of cells proliferated on Col-HA membrane was increased at higher rate compared to that on mPEG-PLGA film during 7 days (Fig. 4). Figure 5 (A) shows that fibroblasts penetrated throughout the Col-HA network, with an increased cell density in the porous membrane. However, very few attached cells were found in a single layer on the surface of the PEG-PLGA film after 7-days incubation (Fig. 5 (B)).

Confocal microscopy observations demonstrated that the actin microfilaments of fibroblasts were polymerized on FN coated Col-HA membranes, uncoated Col-HA membranes, mPEG-PLGA membrane and Interceed™ after incubation for 4 hrs (Fig. 6). It has been reported that the actin fibers in non-muscle cells are involved in forming adhesion plaques

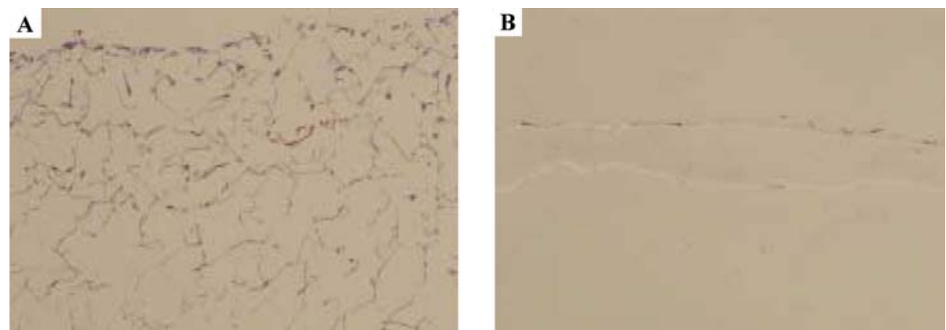

Fig. 5 H&E staining of fibroblasts cultured for 7 days on the collagen-HA matrix (A) or PEG-PLGA film (B) (magnification × 100).

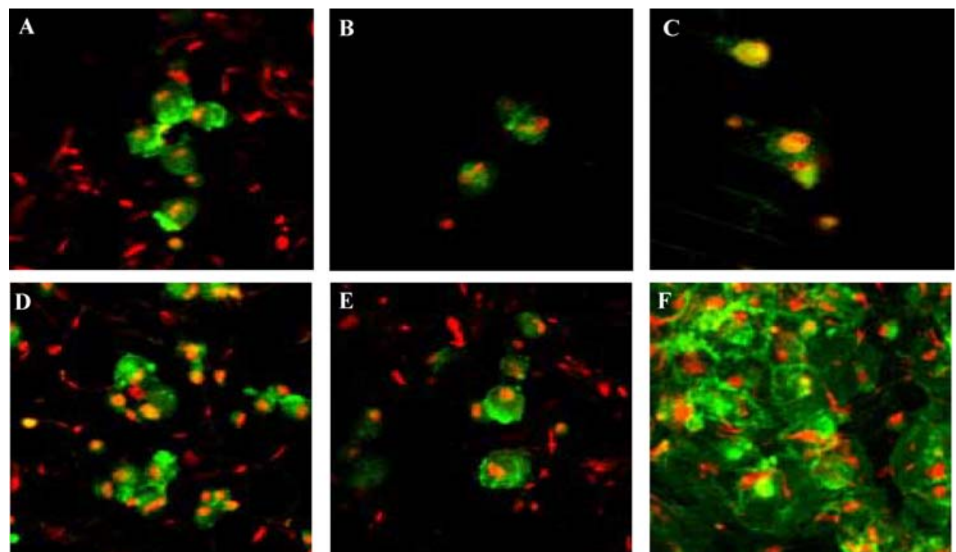

Fig. 6 Confocal microscopic images of FITC-phalloidin stained F-actin (green) and PI stained nucleus (red) in fibroblasts cultured on the collagen-hyaluronic acid membrane (A), mPEG-PLGA film (B), Interceed™ (C) and 30 $\mu g/cm^3$ (D), 50 $\mu g/cm^3$ (E) or 100 $\mu g/cm^3$ (F) fibronectin coated Col-HA membranes.

and cell adhesion to the extracellular matrix (ECM) triggers reorganization of the actin cytoskeleton [18]. The actin cables, which presented in well-spread cells on the FN coated and uncoated Col-HA membranes were formed in association with focal adhesions at the cell periphery. To evaluate the effect of FN coating on the actin filament reorganization, we measured actin-staining intensity of the cells adhered to the FN coated and uncoated membranes. Figure 7 demonstrates that the average pixel intensity of fibroblasts on collagen-HA membrane was 193 ± 3.6 and then significantly increased to 203 ± 1.8 or 205 ± 4.7 when 50 or 100 μg/cm^3 fibronectin was incorporated on the membranes, respectively, indicating the further reorganization of actin microfilaments.

Degradation behavior of the composite membrane

If the majority of fibrous attachments persist for 3 days or longer, fibroblastic proliferation may occur within them, causing adhesion formation. Therefore, the mechanical barriers should stay in place for several days, persist during the critical phases of reepithelialization and undergo absorption following peritoneal healing [19]. In this *in vitro* degradation test, the weight of the composite membrane decreased continuously after being exposed to the PBS at 37°C (Fig. 8). After 2 weeks, about 30% of the initial mass was degraded. The molecular weights of mPEG-PLGA block copolymer were reduced rapidly within the first 3 days, but then decreased gradually throughout the rest of the period, as shown in Fig. 8 B. The subsequent decrease in the degradation rate after the initial degradation may be due to the hydrophilic characteristics of the ethylene glycol segment of the mPEG-PLGA copolymer [20, 21].

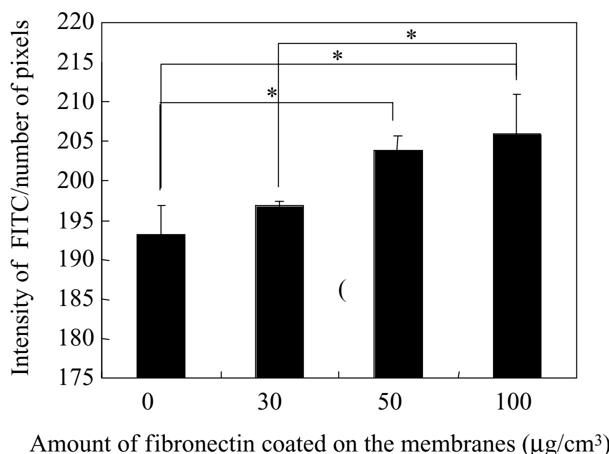

Fig. 7 Fluorescence intensity of F-actin staining, by FITC-phalloidin, in fibroblasts after 4-h of culturing. Acin filaments were further polymerized when 50 or 100 μg/cm^3 of fibronectin was incorporated in the Col-HA membranes compared to 0 or 30 μg/cm^3 of fibronectin incorporated membranes ($^* p < 0.05$).

Preliminary animal study

A porcine model was used to investigate the tissue response to mPEG-PLGA/Col-HA-FN membrane because it has been recognized as a suitable surgical animal model based upon their comparative anatomy and physiology. The peritoneal defect excised on the abdominal wall of a pig is shown in Fig. 9. One week postoperatively, the animal remained in good health and had no wound infection. The gross appearance of repair site is shown in Fig. 10. The Col-HA-FN side of membrane was firmly attached to the musculofascial edges of abdominal wall defect and a layer of mesothelium covered the composite membrane and edges. It has been known that peritoneal mesothelial cells are present at the site of injury within two days, and in the subsequent five days, the defect is covered with mesothelial cell layer [22]. In this preliminary experiment, it was demonstrated that the composite membrane could remain during the critical period of mesothelial repair *in vivo*. However, large-scale *in vivo* study will be required in order to further investigate the efficacy of COL-HA-FN membrane as an anti-adhesive membrane.

At explantation, no inflammatory sign or adverse tissue reaction was seen. Analysis of the section via microscope revealed that the COL-HA-FN membrane was infiltrated with

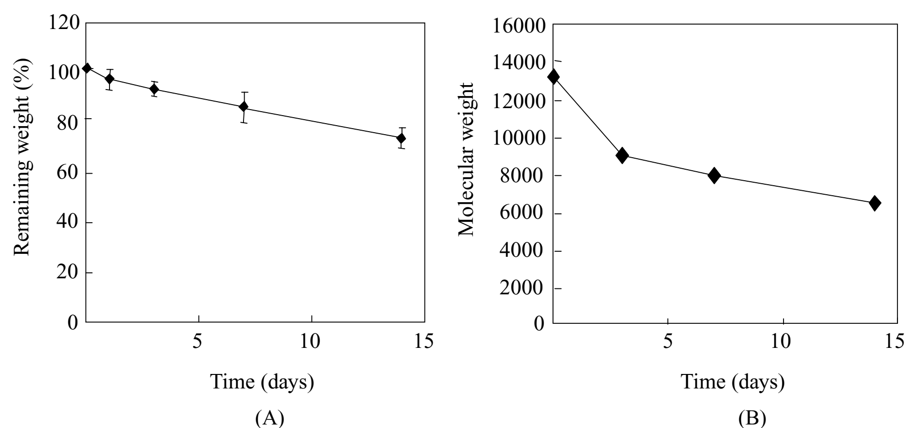

Fig. 8 Degradation profile of the composite membranes, consisting of mPEG-PLGA film and porous collagen-HA membrane: (A) weight loss of membrane, and (B) molecular weight loss of the PEG-PLGA film.

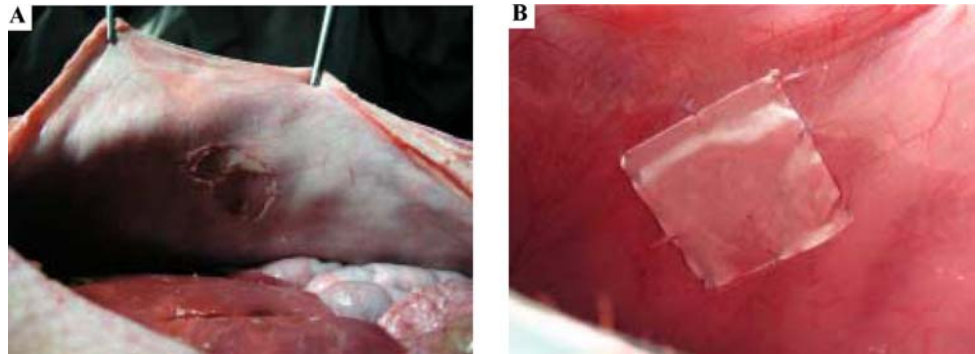

Fig. 9 Gross photographs of peritoneal defect site (A) and a piece of mPEG-PLGA/Col-HA composite membrane applied to the defect (B).

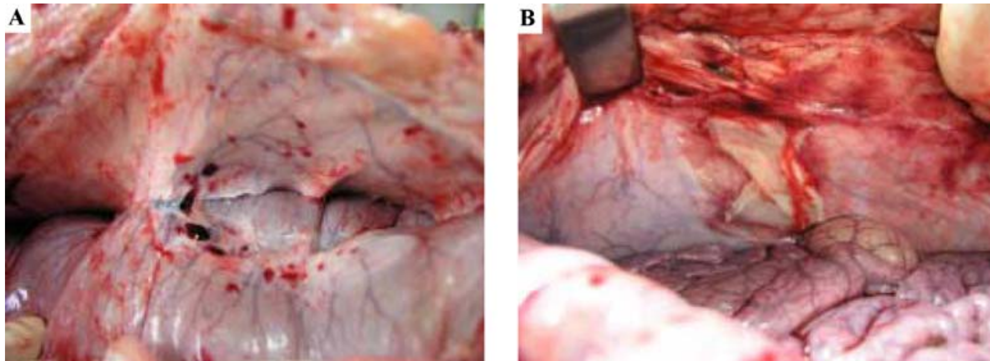

Fig. 10 (A) Photograph of repair sites without treatment at 1 week. There are adhesions to the peritoneum involving the bowel. (B) Photograph of mPEG-PLGA/Col-HA membrane repair at 1 week. There was no adhesion occurrence between the membrane and abdominal structure. The Col-HA-FN side of membrane was firmly attached to the musculofascial edges of abdominal wall defect.

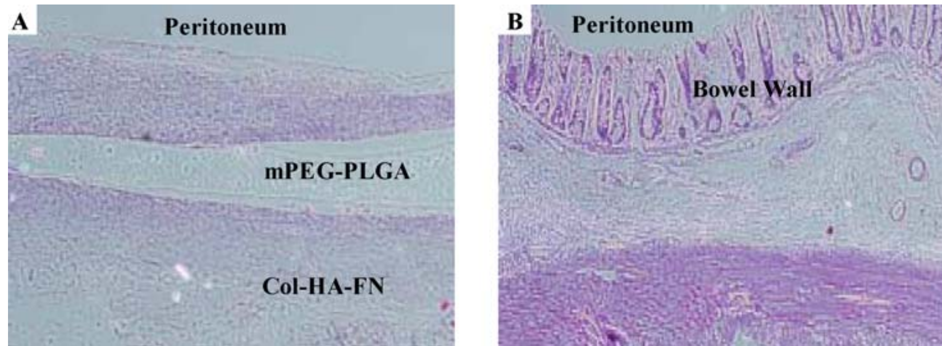

Fig. 11 (A) Hematoxylin and eosin stained cross-section (20×) of mPEG-PLGA/Col-HA membrane repair site at 1 week. The Col-HA membrane side is infiltrated with cells from adjacent tissue and the residual mPEG-PLGA film is surrounded with a continuous tissue layer, which extended below. (B) Hematoxylin and eosin stained cross-section (20 ×) of the non-treated defect site adherent to the bowel.

cells and surrounded with a continuous tissue layer, which extended below the residual mPEG-PLGA film (Fig. 11(A)) and that the non-treated defect site was continuously in direct contact with the abdominal viscera (Fig. 11(B)).

Conclusion

In the present study, the bilayered membrane consisting of mPEG-PLGA flm and Col-HA or Col-HA-FN membrane was prepared as a postoperative adhesion barrier for peritoneal wound healing. In *in vitro* assay, greater number of fibroblasts attached evenly to Col-HA membrane side in comparison with those attached to PLGA film, mPEG-PLGA film side or Interceed^{TM} after 4-h of incubation while the mPEG-PLGA film side showed the lowest cell adhesive properties. 50 or 100 μg/cm^3 FN coated Col-HA membranes increased the formation of adhesion plaques of fibroblasts compared to uncoated Col-HA membrane. *In vitro* degradation test showed that the weight of the composite membrane decreased continuously to 70% of initial mass after 2 weeks. In the preliminary animal study, mPEG-PLGA/Col-HA

membranes with 50 μg/cm^3 FN coating could remain during the critical period of mesothelial repair and did not provoke any inflammation or adverse tissue reaction. Therefore, it is suggested that this composite material which is composed of a Col-HA-FN membrane layer for promoting wound healing, and the mPEG-PLGA film layer for limiting tissue apposition might be useful as an adhesion barrier for peritoneal tissue repair.

Acknowledgment This work was supported by the Korean Research Foundation Grant (KRF-2004-041-D00860).

References

1. D. M. WISEMAN, in "Polymeric site-specific pharmacotherapy" (John Wiley and Sons, Chichester, 1994) p. 369.
2. E. P. GOLDBERG, J. W. BURN and Y. YAACOBI, *Prog. Clin. Biol. Res.* **381** (1993) 191.
3. G. S. DIZEREGA, *Prog. Clin. Biol. Res.* **381** (1993) 1.
4. G. S. DIZEREGA, *Fertil. Steril*. **61** (1994) 219.
5. D. M. WISEMAN, J. R. TROUT, R. R. FRANKLIN and M. P. DIAMOND, *J. Reprod. Med.* **44** (1998) 325.
6. J. M. BECKER, M. T. DAYTON, V. W. FAZIO, D. E. BECK, S. J. STYKER, S. D. WEXNER, B. G. WOLFF, P. L. ROBERTS, E. SMITH, S. A. SWEENEY and M. MOORE, *J. Am. Coll. Surg.* **183** (1996) 297.
7. M. P. DIAMOND, *Fertil. Steril.* **66** (1996) 904.
8. D. B. JOHNS and G. S. DIZEREGA, in "Peritoneal surgery" (Springer-Verlag, New York, 2000) p. 351.
9. D. P. ORGIL, C. CUTLER, J. F. REAGAN, S. M. CARLOW, I. V. YANNA and C. COMPTON, *Plastic. Reconstruct. Surg.* **102** (1988) 423.
10. F. D. MURPHY, D. ORGILL and I. V. YANNAS, *Lab. Invest.* **63** (1990) 305.
11. S. N. PARK, J. C. PARK, H. O. KIM, M. J. SONG and H. SUH, *Biomaterials* **23** (2002) 1205.
12. S. N. PARK, H. J. LEE, K. H. LEE and H. SUH, *Biomaterials* **24** (2003) 1631.
13. H. W. SUNG, I. L. LIANG, C. N. CHEN, R. N. HUANG and H. F. LIANG, *J. Biomed. Mater. Res.* **55** (2001) 538.
14. B. CHEVALLY, N. ABDUL-MALAK and D. HERBAGE, *J. Biomed. Mater. Res.* **49** (2000) 448.
15. S. S. AN, R. E. LAUDADIO, J. LAI, R. A. ROGERS and J. J. FREDBERG, *Am. J. Physiol. Cell. Physiol.* **283** (2002) C797.
16. K. D. PARK, Y. S. KIM, D. K. HAN, Y. H. KIM, E. H. LEE, H. SUH and K. S. CHOI, Biomaterials **19** (1998) 851.
17. P. B. VAN WACHEM, T. BEUGELING, J. FEIJEN, A. BANTIGES, J. P. DETMERS and W. G. VAN AKEN, *Biomaterials* **6** (1985) 403.
18. B. ZIMERMAN, T. VOLBERG and B. GEIGER, *Cell. Motil. Cytoskeleton* **58** (2004) 143.
19. V. GOMEL, B. URMAN and T. GURGAN, *J. Reprod. Med.* **41** (1996) 35.
20. J. T. GARCIA, J. B. FARINA, O. MUNGUIA and M. LLABREAS, *J. Microencapsul.* **16** (1999) 83.
21. H. L. JIANG and K. J. ZHU, *Polym. Int.* **48** (1999) 47.
22. G. S. DIZEREGA, *Eur. J. Surg. Suppl.* **577** (1997) 10.