

Gemcitabine-releasing polymeric films for covered self-expandable metallic stent in treatment of gastrointestinal cancer

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ABSTRACT

Non-vascular drug-eluting stents have been studied for the treatment of gastrointestinal cancer and cancer-related stenosis. In this study, we designed and evaluated a gemcitabine (GEM)-eluting covered nonvascular stent. Polyurethane (PU)/polytetrafluoroethylene (PTFE) film was selected for the drug loading and eluting membrane. The membrane was fabricated by dip-coating on a Teflon bar (ϕ ; 10 mm), air-dried, peeled off and applied to a self-expanding Nitinol stent. Various amounts of poloxamer 407 (PL, Lutrol[®] F127, BASF) (8%, 10%, or 12% of PU by weight) were added to control the release of GEM from membranes. The membrane containing 12% PL (GEM-PU-PL12%) showed the most favourable release properties; 70% of the loaded GEM released within 35 days, including the 35% released during the initial burst. The biological activities of GEM-PU-PL12% were evaluated using human cholangiocarcinoma cells (SK-ChA-1). GEM-PU-PL12% most efficiently inhibited the proliferation of cholangiocarcinoma cells and most highly induced pro-inflammatory cytokines (TNF- α , IL-1 β and IL-12) and p38 MAPKs in the cells. Subtumoural insertion of the GEM-PU-PL12% membrane more efficiently inhibited the growth of CT-26 colon cancer than other membranes. In this study, the GEM-eluting metal stents covered with PU-PL12% showed considerable feasibility for the treatment of malignant gastrointestinal cancer as well as cancer-related stenosis.

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1. Introduction

Nonvascular stents have been used to palliate obstructive symptoms for patients with inoperable tumours in the bile duct as well as gastrointestinal (GI), pulmonary, and urinary tracts (Bezzi et al., 2002; Burstow et al., 2009; Davies et al., 2005; Davis and Nouneh, 2000). Especially for unresectable malignant tumours that in-grow and obstruct the passageway, nonvascular stents are the treatment of choice to keep the tubular way open (Katsanos et al., 2010). The clinical effectiveness of self-expanding metallic non-vascular stents (EMS) has been shown for many years (Dussaillant et al., 1998; Stupart, 2007). First, palliation of obstructive symptoms by introduction of an EMS can avert imperative surgery, leading to improved quality of life for patients. An EMS is an easily implantable device with low complication rates and is more cost-effective than other therapies (Cohen et al., 1994).

However, an EMS works as a simple endoluminal scaffold and allows only mechanical palliation of the obstruction. The

in-growing tumours eventually infiltrate the lumen and again occlude the passageway (Kim et al., 2009a; Machan, 2006). Covered EMSs were developed to overcome this susceptibility of bare EMSs. However, the therapeutic efficacy of covered EMSs is still under question. Ikami et al. reported a covered EMS that showed a superior patency rate to that of bare EMSs, statistically significance 6 months after therapy (Isao et al., 2000). No significant difference was detected between treatments after 6 months. They concluded that covered the EMS contributed to short-term patency but suggested additional anti-cancer therapy is required to improve patency and survival. Restenosis remains as an issue to be resolved (Fischell, 1996; van der Giessen et al., 1996).

Additionally, nonvascular drug-eluting stents (DES) have been recently introduced and evaluated. Nonvascular DESs were designed to release a cancer drug in a controlled manner and to provide both local cancer therapy and prolonged patency. Treatments of unresectable malignant tumours mostly rely on systemic injection of cancer drugs. The injected drugs rapidly reach high plasma concentrations and circulate throughout the whole body; however, the tumour concentration is hardly controlled (Baguley and Finlay, 1995; Paolino et al., 2010). Cancer drugs infiltrate tumours by unexpectedly slow kinetics and are rapidly cleared before attaining a therapeutic concentration in tumour tissues. Local cancer

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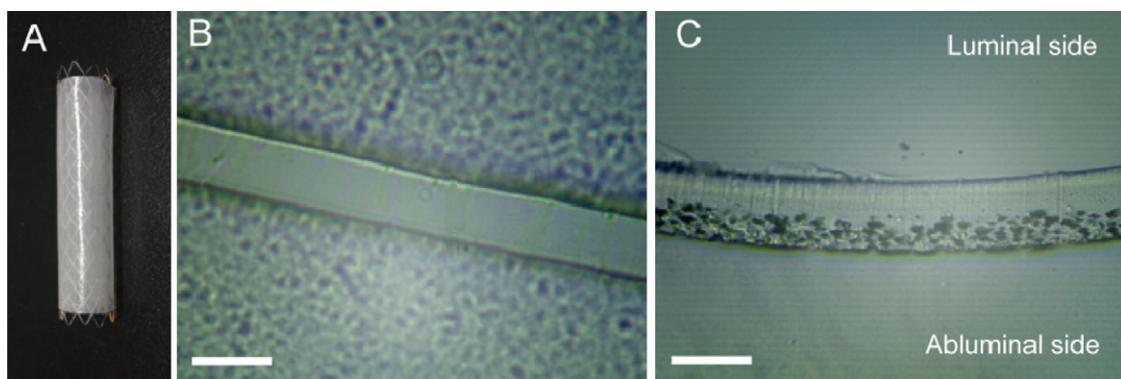


Fig. 1. GEM-eluting, self-expandable non-vascular stent. (A) Photo images of stent. (B and C) Cross-sectional microscopic view of the PU-PL12% (B) and GEM-PU-PL12% (C) membrane. GEM crystals were observed on the tumour side of GEM-PU-PL12% and lead to the initial burst release of GEM observed during the release study. Nucleation and then crystallisation of GEM seemed to be derived from solvent evaporation during the film formation. Scale bar corresponds to 50 μ m.

treatment rather than systemic treatment could be a more reasonable therapy especially for un-resectable tumours (Shiino et al., 1994). The released drug locally accumulates in cancer tissues without systemic exposure, inducing apoptosis of cancer cells. Non-vascular DESs for gastrointestinal cancer were investigated based on the above-mentioned backgrounds, and a covering membrane was functionally designed for a drug loading and releasing film. The physicochemical and compositional properties of the polymer membrane critically affect the release of drug (Kim et al., 2009b), and scientists manipulate polymer membrane properties to achieve a homogeneous and sustained drug release during the patency.

In this study, we used gemcitabine (GEM) and polyurethane (PU) as a model drug and film-forming polymer, respectively, for the design of a nonvascular DES. GEM is used to treat non-small-cell lung cancer (Hoang et al., 2003), pancreatic cancer (Carmichael et al., 1996), metastatic breast cancer (Carmichael et al., 1995), and ovarian cancer (Fruscella et al., 2003).

GEM also showed a good therapeutic efficacy for the treatment of bile duct cancer in combination chemotherapy with fluorouracil. EMSs have been most commonly applied to secure the passage of bile acid. We designed a GEM-eluting DES that can specifically target bile duct cancer. Our GEM-releasing membrane has a double-layered structure, composed of PTFE for the backing layer and polyurethane for the GEM-loading layer. Polyurethane, silicone, and polytetrafluoroethylene (PTFE) have been selected for the covering membrane because of their superior resistance and strength against severe gastrointestinal circumstance (Bezzi et al., 2002; Isayama et al., 2004; Thurnher et al., 1996). However, prolonged GEM release more than 2 weeks is hardly attainable due to the high water-solubility of GEM (15.3 mg/mL) (Pili et al., 2009). Thus, GEM was nano-granulated to get the more favourable release profiles. Mixture of tetrahydrofuran (THF) and ethanol was selected as a dipping solution which can maintain GEM in crystal form in dipping solution. We expect the release rate of GEM nano-granulated is slower than that of free GEM because of the smaller surface area of GEM nano-granulated (Scheme 1). Also, Lutrol® F127 (PL) was selected as a release modifier. The amount of Lutrol® F-127 was adjusted to 0%, 8%, 10% or 12% (w/w) of PU by weight and named GEM-PU, GEM-PU-PL8%, GEM-PU-PL10%, and GEM-PU-PL12%, respectively.

The membranes were fabricated using the dipping method following our previous report (Moon et al., 2011). In this study, GEM release profiles, surface morphologies of its membranes, cytotoxicities of the release of GEM from the membranes, expression of pro-inflammatory cytokines and anti-cancer efficacy against CT-26 colon tumour-bearing mice were evaluated.

2. Materials and methods

2.1. Materials

Gemcitabine HCl (GEM) was provided by Dong-A Pharmaceutical Co. (Seoul, South Korea). Self-expandable non-vascular metallic stents (EMS), PTFE membranes and polyurethane (Pelletthane® 2363-80AE) were kindly supplied by Taewoong Medical Co. (Goyang-si, South Korea). Poloxamer 407 (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)) was purchased from BASF (Ludwigshafen, Germany) in commercial form (Lutrol® F127; PL). Tetrahydrofuran (THF) and were obtained from Sigma Co. (St. Louis, MO, USA). All reagents used were of extra pure reagent grade without the need for further purification.

2.2. Preparation of the GEM-eluting membrane for the EMS

GEM-eluting membranes were fabricated using the dip coating method. Polyurethane (PU), poloxamer 407 (PL, Lutrol® F-127) and GEM were dissolved in 8.5 mL of tetrahydrofuran (THF), 1.0 mL of THF and 1.0 mL of ethanol (75%, v/v, in water), respectively. The solutions were then mixed together, homogenised at 15,000 rpm (Ultra-turrax T-25®, IKA, Germany) for 2 min and used as the dipping solution.

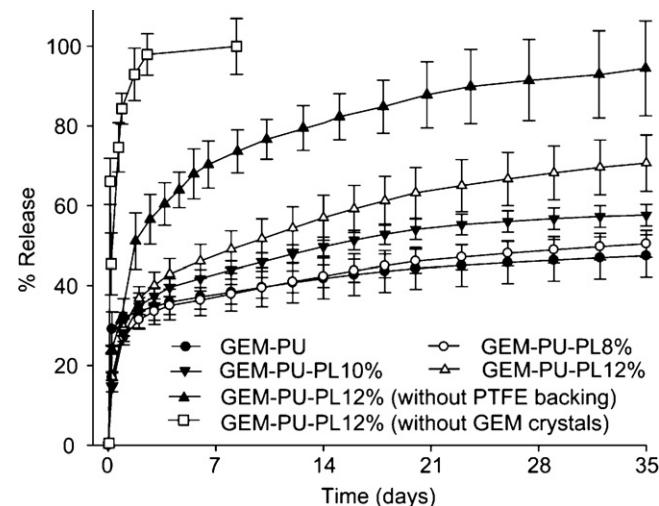
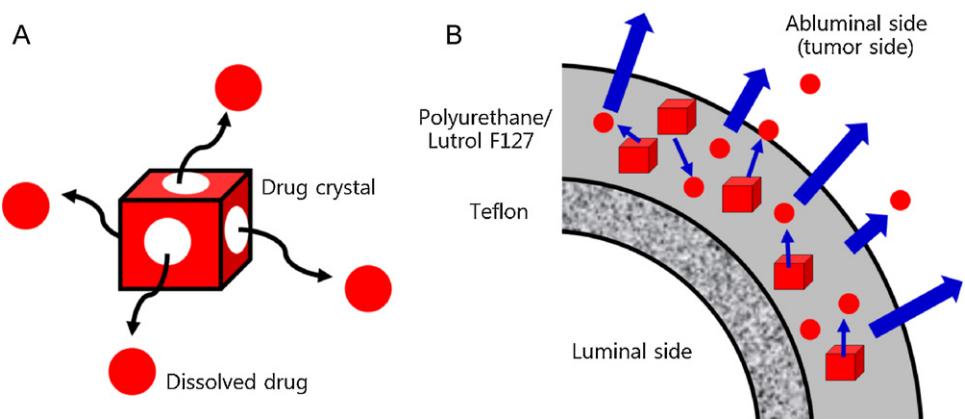


Fig. 2. Release of GEM from PU membrane (PU) and polyurethane-Lutrol® F127 membranes (PU-PL). Lutrol® F127 was applied by weight percentage of PU. GEM-PU-PL12% showed the most controlled release of GEM with an initial burst release of 35% of the total loaded GEM, followed by a sustained release of an additional 35% ($n=3$).



Scheme 1. Release of gemcitabine (GEM) in nano-granulated states. (A) The proposed dissolving stage of GEM crystals in polymeric membranes. (B) Release of GEM in nano-granulated states from PTFE-polyurethane membrane.

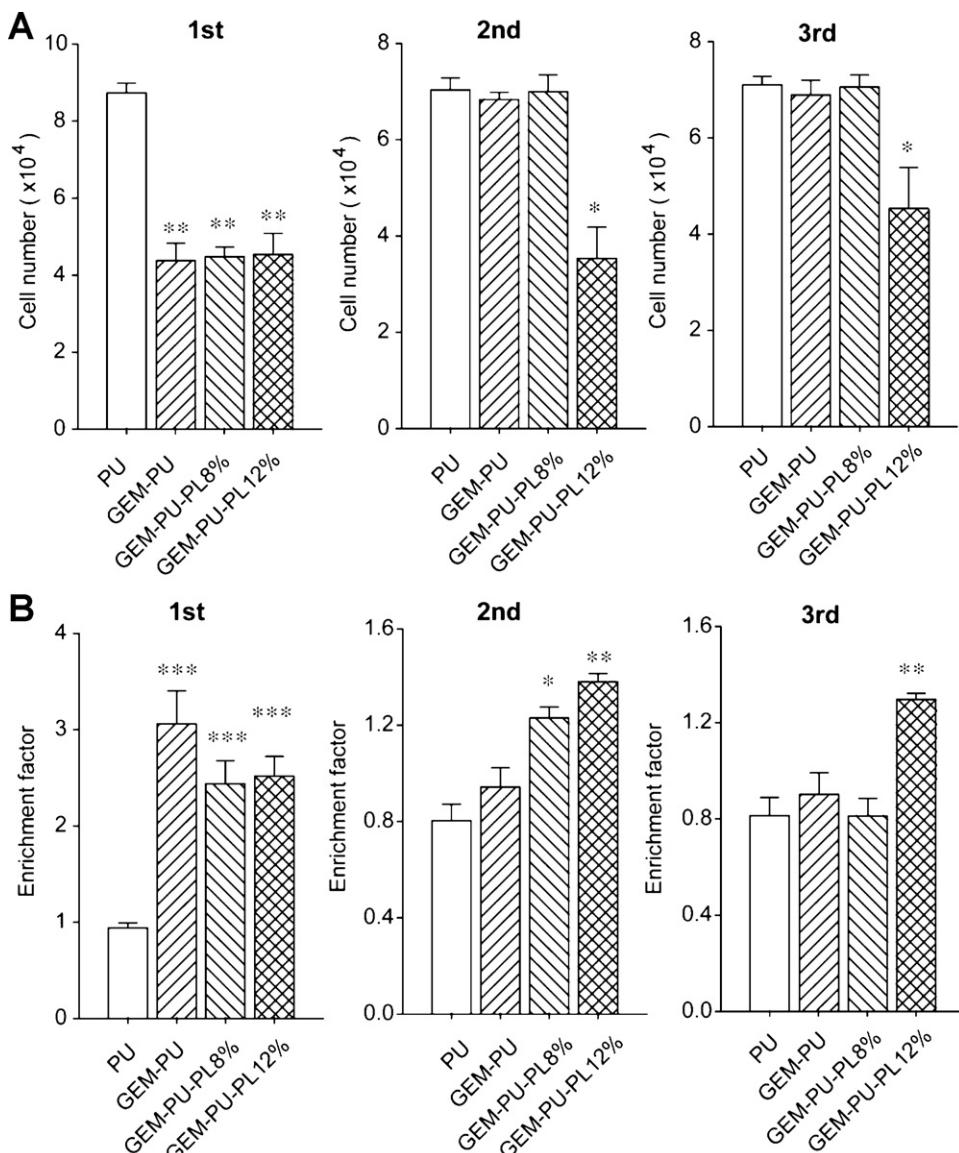


Fig. 3. Inhibition of cell proliferation due to the released GEM from the GEM-eluting membranes (PU, GEM-PU, GEM-PU-PL8% and GEM-PU-PL12%). The viable cell number (A) and the enrichment factor (B) of SK-ChA-1 cells were estimated after treatment with the membrane-released GEM. GEM-PU-PL12%, which released GEM in a steady manner for 35 days, showed the most proficient cell-growth inhibition (**p < 0.001, *p < 0.01, *p < 0.05, as compared with PU).

A PTFE membrane was used as the primary layer. The Teflon bar (ϕ : 10 mm) covered with PTFE membrane was submerged to a dipping solution, withdrawn, and air-dried for over 5 h. Dip-coating was performed three times, and the final dipping was conducted with PU solution without Lutrol® F-127 and GEM. The air-dried cylindrical membranes were carefully peeled off the Teflon bar and applied to the EMS. The amount of Lutrol® F-127 was adjusted to 0%, 8%, 10% and 12% (w/w) of PU by weight and named as GEM-PU, GEM-PU-PL8%, GEM-PU-PL10%, and GEM-PU-PL12%, respectively. Additionally, polyurethane membrane without GEM and Lutrol® F127, named PU, was fabricated via the same method for the *in vivo* control test.

The integrities of membranes were observed. Membranes were embedded in a paraffin block, sliced into 5 μ m sections with a microtome and inspected using an optical microscope.

The amount of GEM in each membrane was estimated using HPLC (Alliance 2695 system, waters, USA) after serial dissolution of the membrane in THF and water. GEM was analysed at room temperature using a Thermo Scientific Hypersil C₁₈ column (150 mm \times 4.6 mm, Thermo Electron Co., UK). The mobile phase consisting of water/acetonitrile (95:5, v/v) was delivered at a flow rate of 0.5 mL/min. GEM was detected at 269 nm.

2.3. In vitro release study

The GEM-eluting membrane covered EMSs were each placed in a 50 mL conical tube which was containing 40 mL of 0.01 M phosphate buffered saline (PBS) and horizontally shaken in a water

bath at 37 °C and 50 rpm. Five millilitres of PBS from each tube was collected for analysis at predetermined time intervals and replaced with the same volume of fresh PBS. The amount of GEM was determined using a UV spectrophotometer (UV-1601, Shimazu Co., Japan) at 266 nm.

2.4. Cell culture

SK-ChA-1 (human cholangiocarcinoma cell) and CT-26 (murine colorectal carcinoma cell) were selected for *in vivo* and *in vitro* estimation of therapeutic efficacies. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (WelGENE Biopharmaceuticals, Daegu, Korea) containing 10% foetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μ g/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO₂.

2.5. In vitro cytotoxicities of the released GEM

GEM-released medium was periodically recovered, filtered, diluted with complete media, and introduced to SK-ChA-1 cells. After 24 h of treatment, cytotoxicities of the released GEM were estimated based on the number of viable cells and the cellular DNA fragmentation. Cells were detached with trypsin/EDTA and resuspended in test tubes. The number of viable cells was counted using a haemocytometer after staining dead cells with trypan blue. In parallel, cellular DNA fragmentation was determined using a cell death detection ELISA kit (Cell Death Detection ELISA^{PLUS}, Roche Applied Science, USA) according to the manufacturer's introduction. DNA

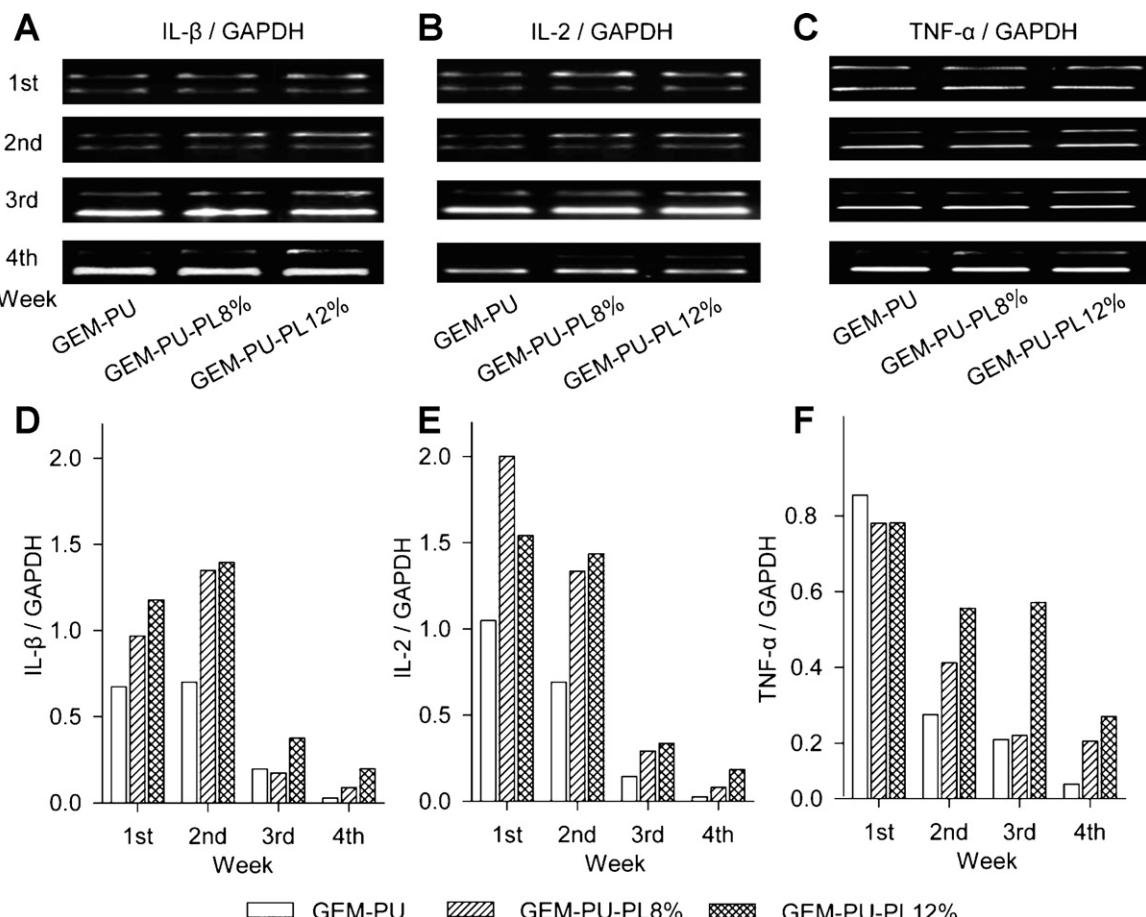


Fig. 4. Cellular mRNA expression of pro-inflammatory cytokines. (A, B and C) RT-PCR showing cellular expression of IL-1 β (A), IL-2 (B) and TNF- α (C) in SK-ChA-1 cells under treatment with GEM-eluting membranes (GEM-PU, GEM-PU-PL8% and GEM-PU-PL12%). (D, E and F) Relative intensities of cytokines to GAPDH (D, IL-1 β ; E, IL-2; and F, TNF- α). The intensities were measured using Image J.

fragmentation, which was induced by GEM, was expressed with an enrichment of histone-associated mono- and oligo-nucleosomes released into the cytoplasm. The enrichment factor represents the amount of histone-associated mono- and oligo-nucleosomes released into the cytoplasm. The enrichment factor was calculated with an absorbance at 405 nm.

2.6. Cellular expression of pro-inflammatory cytokines

The GEM solution was recovered from the release study by the same method as the previous cytotoxicity study and was applied to SK-ChA-1 cells. After 24 h of treatment with GEM solution, intracellular levels of tumour necrosis factor (TNF- α) and interleukins (IL-1 β and IL-12) of SK-ChA-1 cells were evaluated. Total RNA was isolated using an RNeasy mini-kit (Qiagen, Fremont, CA, USA) according to the manufacturer's instructions. Aliquots of RNA were reverse-transcribed to cDNA using the AdvantageTM RT-for-PCR kit from Clontech. Primers and probes were constructed based on the reported cDNA sequences for human TNF- α , IL-1 β , IL-12, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), shown in Table 1. PCR was performed using the following settings for the air thermocycler: a denaturing temperature of 94 °C for 1 min, an annealing temperature of 62 °C for 30 s, and an elongation temperature of 72 °C for 45 s for the first 36 cycles and finally an elongation temperature of 72 °C for 10 min. Following the reaction, the amplified products were removed from the tubes and run on a 2% agarose gel.

2.7. Western blotting of p38 MAPKs in SK-ChA-1 cells

Cells were washed with phosphate-buffered saline (PBS) and lysed for 1 h at 4 °C in a lysis buffer. Total cell lysates were prepared in a lysis buffer (20 mM Tris buffer), 150 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM sodium orthovanadate, 1 μ g/mL leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Total cell protein concentration was determined using a protein assay kit (BIO-RAD). Twenty micrograms of cell lysates were loaded and separated on 8% SDS polyacrylamide, and western blotting was performed using the indicated antibodies (Cell Signalling Technology, Inc., MA) (Rube et al., 2004).

2.8. In vivo inhibition of subcutaneous colon cancer growth

In vivo inhibition of cancer growth by the GEM-eluting stent membranes was evaluated in CT-26 colon carcinoma-bearing mice. CT-26 metastatic murine colon cells were subcutaneously injected into the flanks of male BALB/c mice (Orient Co., Korea) at a concentration of 1.0×10^6 cells. When tumours reached an average diameter of ~ 6 mm, GEM-eluting membranes were surgically implanted under the tumours. A total of 150 μ g of GEM was incorporated into each membrane. Twenty-five mice were randomly divided into five groups as follows: (1) non-treated, (2) PU, (3)

Table 1
Oligonucleotide sequences of the primers used for amplification of IL-1 β , IL-12 and TNF- α .

Name	Primer	Sequence
TNF- α	Sense	5'-AGAAGGAAACAGACCAAGAC-3'
	Antisense	5'-GGGAAAGAAATCATTCAACCA-3'
IL-1 β	Sense	5'-AGAACGTTCCACCAATACTC-3'
	Antisense	5'-AGCACCTAGTTGTAAGGAAG-3'
IL-12	Sense	5'-AAGGAGGGAGGTCTAACG-3'
	Antisense	5'-GTACTCCAGCTGACCTCA-3'
GAPDH	Sense	5'-ACCACAGTCCATGCCATCAC-3'
	Antisense	5'-TCCACCACCTGTGCTGTA-3'

GEM-PU, (4) GEM-PU-PL8%, and (5) GEM-PU-PL12%. Tumour volume and the weights of the mice were periodically monitored. Two perpendicular diameters of the tumours were measured using Vernier callipers (Mitutoyo Co., Japan), and the volume was calculated using the formula $V = (a \times (b \times b))/2$, where a is the largest diameter and b is the smallest.

3. Results and discussion

3.1. GEM-PU-PL membrane covered metal stent

Our GEM-eluting membrane for a non-vascular stent was basically composed of primary PTFE and secondary PU layers. PTFE was employed as a supporting layer, and PU was designated as a GEM-loading layer. PL was applied as a release modifier and solubiliser of GEM, and the amount of PL was varied to 0%, 8%, 10% or 12% (w/w) of PU. Fig. 1 displays the structure of our covered stent. Cross-sectional images of the GEM-PU-PL membrane show crystalline GEM located in the middle of membrane, while PU-PL membrane without GEM showed no crystalline substance in the middle of membrane (Fig. 1B and C). Nucleation and then crystallisation of GEM seemed to be caused by solvent evaporation during the film formation (Zilberman et al., 2006). GEM-PU-PL12% produced a membrane thickness of about 50–60 μ m. Overall GEM loading was approximately 2.33 ± 0.05 mg per membrane.

3.2. In vitro release of GEM

As mentioned above, the release of GEM from PU membrane is fast from the high water-solubility of GEM. PU membrane which is non-biodegradable and hydrophobic can retard the release of GEM. Indeed, the fast release and high initial burst (up to 70% of

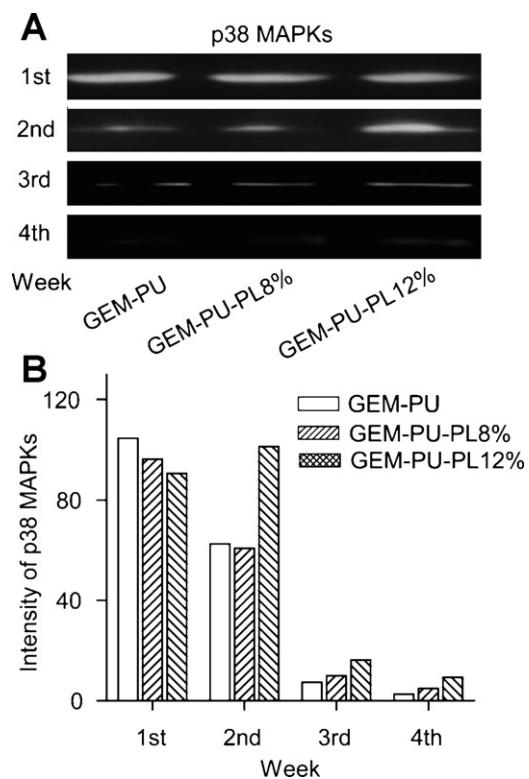


Fig. 5. Expression profiles of apoptotic p39 MAPKs. (A) Western blotting showing cellular expression of p39 MAPKs in SK-ChA-1 cells during the treatment with GEM-eluting membranes (GEM-PU, GEM-PU-PL8% or GEM-PU-PL12%). (B) Intensities of expressed p39 MAPKs. The intensities were measured using Image J.

the loaded dose) of GEM from PU membrane was reported by our group and proved again in this study (Fig. 2) (Moon et al., 2011), while PU seemed to prevent water penetration and inhibited the release of GEM.

In order to improve the release behaviour of GEM from PU membrane, nano-granulated GEM was used. The release behaviour of GEM nano-granulated from the GEM-PU membrane was investigated under simulated physiological conditions (PBS, pH 7.4, 37 °C). The initial burst of GEM nano-granulated from GEM-PU membrane dramatically decreased as expected (Fig. 2). This result indicates that the granulated shape (reduced surface area) of hydrophilic drug is useful for long-term drug delivery. Unfortunately, GEM only released an additional 5% of GEM during the 33 days of release following the initial burst release of 33%. Thus, PL is employed as an additional ingredient to facilitate the release of GEM from the membrane. PL has a thermo-sensitivity around 30 °C. Although, PL in THF containing PU is highly stretched form, the polymer may be entangled in the condition of water at 37 °C. The phenomenon may lead to make channels and holes in the membrane for facilitation of water-penetration.

As shown in Fig. 2, all GEM-PU-PL membranes showed initial bursts of drug release around 30–40% of the total loaded GEM. GEM-PU-PL12% released GEM in the most controlled manner. In total, 70% of the loaded GEM was released, including the burst release of 35%. After the initial burst, 23.3 µg of GEM was released per day for the 35 days of the release study. The result means that

PL acted as a water-diffusion enhancer and seemed to contribute to the controlled release of GEM after the initial burst. However, increasing the amount of PL above 15% caused an initial burst of up to 60% of the loaded dose (data not shown).

Our data also showed that the primary PTFE backing affected the release of GEM. Primary PTFE backing was applied to enhance the mechanical strength of membrane and to obtain a one-directional release of GEM towards the mucosal side of tissue where gastrointestinal cancer, such as colon cancer or bile-duct cancer, may grow. The GEM-PU-PL12% film without the PTFE backing layer showed a much higher initial burst release and a rapid execution of secondary release.

3.3. In vitro biological activities of GEM-PU-PL films

As shown in the previous release study, prolonged release of GEM may inhibit growth of cancer, thus preventing stenosis of the gastrointestinal tract. Biological activities of the released GEM were evaluated based on the cell proliferation, DNA fragmentation and expression of pro-inflammatory cytokines. The human cholangiocarcinoma cell line (SK-ChA-1) was treated with the recovered release medium after the proper dilution. Fig. 3 shows that only GEM-PU-PL12% efficiently inhibited the proliferation of the cholangiocarcinoma cells, which was estimated based on the cell number for the first 3 weeks of release. The enrichment factor also

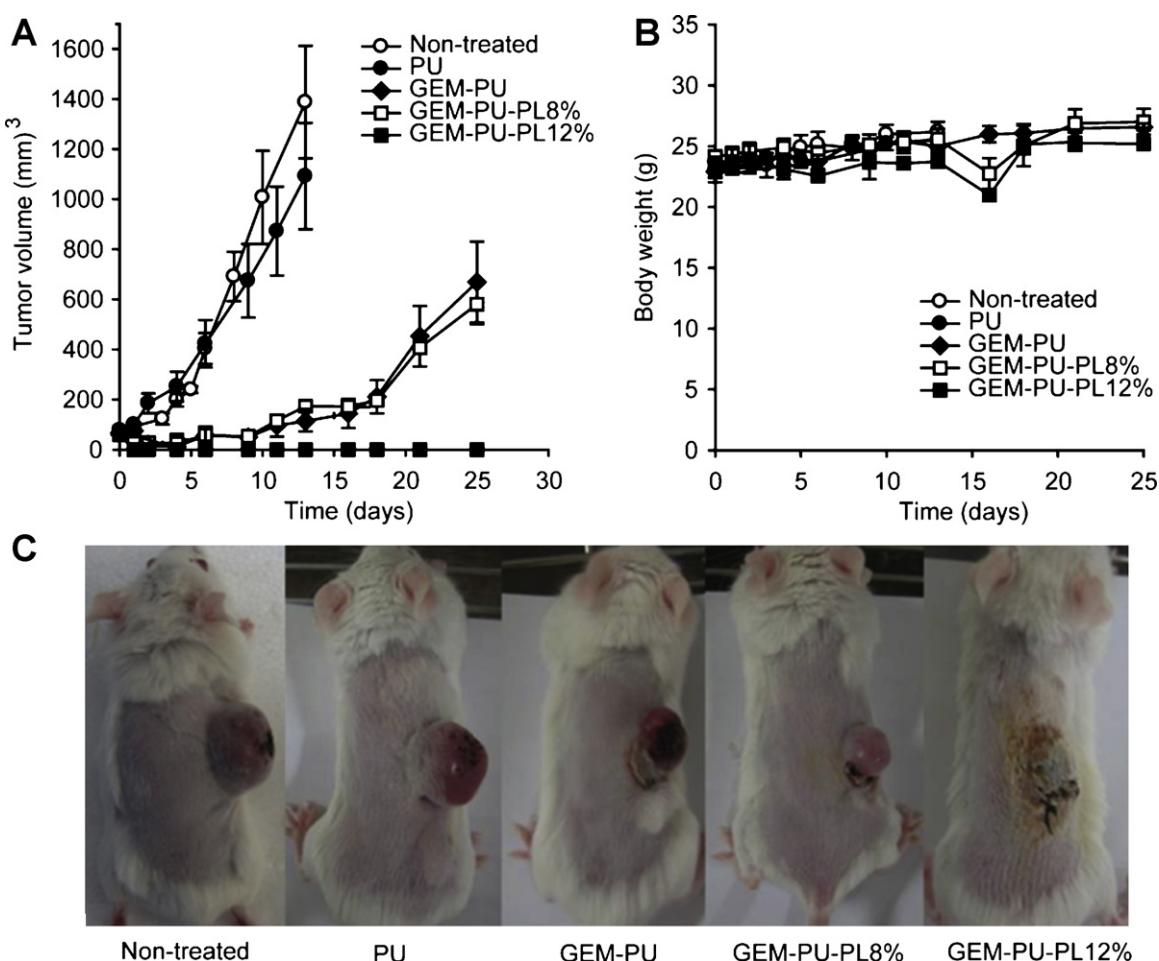


Fig. 6. In vivo inhibition of tumour growth. (A) Tumour-growth curves. (B) Body weight changes of mice during the treatments. There were no detectable serious body changes, suggesting no systemic side effects. (C) Photo-images of CT-26 xenograft mice at the end of study. Stent membranes (PU, GEM-PU, GEM-PU-PL8% or GEM-PU12%) were inserted at the subtumoural site, and the volumes were calculated using the formula $V = (a \times (b \times b))/2$, where a is the largest diameter and b is the smallest. Subcutaneous CT-26 tumours inserted with GEM-PU12% showed total regression (A and C).

showed that GEM-PU-PL12% maintained cytotoxic activities for at least 3 weeks. GEM is a nucleoside analogue that contains fluorine atoms instead of hydrogen atoms on the 2' carbon of deoxycytidine. The triphosphate analogue of gemcitabine intercalates DNA blocks, arrests DNA replication and induces apoptosis (Hui and Reitz, 1997). Activated endogenous endonucleases on the apoptotic pathway increase the portion of DNA fragments. Other films showed an absence or nearly all biological activity after 2 weeks of release.

The stability and biological activity of the released GEM during 30 days of delivery time were assessed based on the expression level of pro-inflammatory cytokines (TNF- α , IL-1 β and IL-12) in SK-ChA-1 cells. The mRNA expression of TNF- α , IL-1 β and IL-12 of SK-ChA-1 cells treated with the released GEM is shown in Fig. 4. The relative intensities of cytokines to GAPDH were measured using Image J. Fig. 4 shows that pro-inflammatory cytokines, especially TNF- α , were up-regulated in cells treated with the released GEM from GEM-PU-PL12%, compared to other films. Furthermore, the intensities were maintained for at least 30 days, suggesting the sustained release of GEM from GEM-PU-PL12% might be functionally active for 30 days of delivery time. p38 MAPKs are activated by environmental stress and inflammatory cytokines and are involved in cell apoptosis. The cellular expression of p38 MAPKs was influenced by the release of GEM and also correlated highly with the expression levels of TNF- α , IL-1 β and IL-12 (Fig. 5).

3.4. In vivo efficacy of GEM-PU-PL membrane on tumour growth

The in vivo antitumour activities of GEM-PU-PL films against CT-26 murine colorectal tumours are shown in Fig. 6. Untreated or PU membrane-inserted tumours grew exponentially. However, the growth of GEM-PU-PL film-inserted tumours was significantly inhibited, and GEM-PU-PL12% totally inhibited tumour-growth. The average tumour volumes at the end of the study were $1385 \pm 219 \text{ mm}^3$ for the non-treated group, $1089 \pm 209 \text{ mm}^3$ for the PU film-inserted group and $577 \pm 163 \text{ mm}^3$ for the GEM-PU-PL8% film-inserted group. Subcutaneous tumours inserted with GEM-PU-PL12% film exhibited total regression (Fig. 6A and C). The prolonged release of GEM with an appropriate loading dose, most-effectively accomplished by GEM-PU-PL12% film as shown in the release study, was also most effective in the treatment of an in vivo colon cancer model. No serious adverse events, such as weight loss, ruffling of fur or decrease of food consumption, were observed during the study.

A targeted drug delivery could improve therapeutic efficiency either by increasing efficacy or by reducing side effects. Non-vascular DES for the treatment of gastrointestinal tumours showed the typical characteristics of local drug delivery systems (Gallia et al., 2005; Wang et al., 2007): a concentration of the therapeutic agent at the tumour site, an enhancement of the drug-exposure time to the tumour and a reduction of the systemic exposures.

4. Conclusion

In this study, we evaluated the feasibility of a GEM-eluting covered stent for the treatment of gastro-intestinal cancer and cancer-related stenosis. A PU-PL membrane was selected for GEM-loading and for the stent-covering film, which was fabricated by dip-coating. The release of GEM from the stent was sustained for 30 days, and the release kinetics were affected by the content of PL. Subcutaneous CT-26 colon tumours completely regressed following the treatment with the GEM-PU-PL12% film. These results suggest that local treatment of gastro-intestinal cancer with GEM-eluting stent can be another choice for treatment of gastro-intestinal cancer and stenosis.

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