An acetylated polysaccharide-PTFE membrane-covered stent for the delivery of gemcitabine for treatment of gastrointestinal cancer and related stenosis

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Abstract

Gemcitabine (Gem) eluting metal stents were prepared for potential application as drug delivery systems for localized treatment of malignant tumors. Pullulan, a natural polysaccharide, was chemically acetylated (pullulan acetate; PA) by different degrees (1.18, 1.71, and 2.10 acetyl groups per glucose unit of pullulan), layered on polytetrafluoroethylene (PTFE), and applied as part of a Gem-loaded controlled-release membrane for drug-eluting non-vascular stents. PA with a higher degree of acetylation had greater drug-loading capacity with more extended release of Gem over 30 days. The released Gem accumulated in CT-26 colon cancer without systemic exposure inducing total regression of tumors. The long-term biological activity of the released Gem and apoptosis of tumor tissues following localized delivery were confirmed by annexin V binding assays and histology. The controlled release of Gem from PA-PTFE covered drug-eluting stents (DES) may increase the patency of these stents for the treatment of malignant gastrointestinal cancer as well as cancer-related stenosis.

Keywords:
Nonvascular drug-eluting stents (DES) gemcitabine covered stents pullulan gastrointestinal cancer

1. Introduction

The advent of cardiovascular stents was a major achievement in the treatment of obstructive coronary artery disease [1,2]. However, the incidence of hyperplastic restenosis caused by proliferation and migration of vascular smooth muscle cells presented another problem. The recent introduction of drug-eluting stents (DES) provided a promising breakthrough with regard to the issue of restenosis [2]. Based on clinical success and experiences in the cardiovascular area, stent technology has continued to rapidly expand into other therapeutic areas [3,4]. Non-vascular stents have been investigated as non-invasive alternatives to surgery for treatment of benign and malignant esophageal, gastrointestinal, and bile duct strictures in particular. However, these non-vascular stents also are subject to restenosis [3]. Benign hyperplasia can develop in response to interactions between the foreign stent materials and human tissues similar to what occurs in the vascular restenosis [5]. Malignant tissues (i.e., bile duct cancer, colon cancer, etc), which are the major sites for non-vascular stent applications, overgrow and ultimately infiltrate the lumen, resulting in occlusion of the passageway. The first generation of non-vascular stents was made of hard plastic and used for obstructive esophageal cancers. Self-expandable metallic stents (EMS) covered by a polymeric membrane using polyurethane, silicone, and polytetrafluoroethylene (PTFE) were developed in the 1990s [6–8]. The covered EMS are easy to insert and rapidly improve symptoms with relatively few complications [9,10]. However, continuously growing tumors compromise the patency of the stents, causing re-obstruction of the gastrointestinal tract [11,12]. Consequently, non-vascular DESs were introduced and evaluated [8,9]. The first human trial of non-vascular DES involved 21 patients with unresectable adenomatous esophageal cancer [13]. A total of 11 patients received tantalum Strecker stents coated with ethylene vinyl acetate (EVA) and 3% paclitaxel, while 10 were treated with uncoated Strecker stents. Unfortunately, this study failed to demonstrate that non-vascular DES were effective in preventing tumor ingrowth, but did prove the lack of complications associated with this device and provided possibilities for further development. Recently, an animal experiment [14] and a human pilot study [15] involving the application of DES for bile duct cancer were reported, and a preclinical comparative study of conventional non-drug-coated stents and DES is in progress. Therefore, we anticipate in the near future, that non-vascular DES will become widely used to treat unresectable malignant obstructions, although commercially available DES for non-vascular use has not yet been developed.
In this study, we designed Gemcitabine (Gem)-eluting EMS for treatment of gastrointestinal cancer and cancer-related stenosis. To attain the extended release of Gem, a double-layered polymeric membrane was designed as the stent-covering material. Pullulan acetate (PA) was applied as the drug-loading controlled-release membrane, with PTFE as the primary membrane. Pullulan is a neutral glucan (similar to amylose, dextran, and cellulose), which is an edible, and biodegradable polymer without any local and systemic toxicities [16–19]. The major commercial use of pullulan is for manufacturing edible films. Thus, we studied the feasibility of using PA as a stent-covering membrane which can control the release of cancer drugs, especially hydrophilic drugs, at local sites (i.e., bile duct and colon). An acetyl group was chemically introduced to pullulan to increase the solvent compatibilities and the film-forming properties. Pullulan is only soluble in water, not in organic solvents; however, acetylated pullulan (PA; pullulan acetate) dissolves well in volatile organic solvents (i.e., methylene chloride and tetrahydrofuran) and is easy to prepare as a stent-covering film. Additionally, in an effort to determine whether or not PA is effective in a therapeutic drug delivery system, Gem was employed as a typical water-soluble cancer drug. Gem, 2’2’-difluoro-2’-deoxycytidine (dFdCyd) is effective in the treatment of solid tumors [20,21] including ovarian [22], colon [23], breast [24], bladder [25] and small-cell lung cancer [26]. It has also been used in the treatment of pancreatic cancer [27], in combination chemotherapy treatment of non-small-cell lung cancer; [28] and for leukemia. Although Gem works as an excellent anti-cancer drug with wide applications and high efficacy, pharmaceutical applications have been restricted due to its high water-solubility (15.3 mg/mL) [29]. Various delivery systems such as liposomes and nanoparticles have been investigated, but to date they have not proved effective for local or systemic controlled delivery of Gem [27].

PA-layered PTFE membranes were fabricated using the dipping method. Surface morphologies, Gem release profiles, and cytotoxicities of Gem-loaded PA membranes were observed. In vivo testing was also carried out to evaluate anti-cancer efficacy using CT-26 colon tumor-bearing mice for potential stent placement applications.

2. Materials and methods

2.1. Materials

Pullulan (Mw 100,000) was purchased from Hayashibara Co., Japan. Pyridine, acetic anhydride, formamide, and dimethyl sulfoxide (DMSO) were obtained from Sigma Co. (St. Louis, MO, USA). Gem HCl was provided by Dong-A Pharmaceutical Co. (Seoul, South Korea). All reagents used were of extra pure reagent grade without any need for further purification. Self-expandable non-vascular metallic stents (EMS) and PTFE membranes were supplied by Taewoong Medical Co. (Goyang-si, South Korea).

2.2. Acetylation of pullulan

The pullulan acetylation procedure previously reported by our group [30] was performed as follows: 2 g of pullulan was dissolved in 20 mL of formamide at 50 °C, 7 mL of pyridine was added and vigorously stirred for 1 h at room temperature, and various amounts of acetic anhydride (3, 7, and 15 mL) were added to obtain different degrees of acetylation. This mixture was reacted at 54 °C for 24 h. PA was obtained after precipitation from 200 mL of water. To remove impurities, the powder was re-dissolved in 15 mL of DMSO and re-precipitated with 400 mL of ethanol. The precipitant was dried for 3–4 h at 40 °C in a vacuum oven. The synthesized PA was identified by Fourier transform infrared spectroscopy (FT-IR) and 1H nuclear magnetic resonance (NMR). The degree of acetylation of PA was determined based on the integration ratio of peaks at 1.8–2.2 ppm (COCH3) and 4.5–5.8 ppm (sugar ring) of 1H-NMR (Fig. S2) [31].

2.3. Design of PA-PTFE covered Gem-eluting metallic stents

PA-PTFE covered Gem-eluting (Gem-PA-PTFE) stents were fabricated using the dip coating technique. PA (385 mg) and Gem (20 mg) were dissolved in 5 mL of

Fig. 1. Photo images of pullulan acetate polytetrafluoroethylene (PA-PTFE)-covered, gemcitabine (Gem)-eluting, self-expandable non-vascular stent (a), scanning electron micrograph of PTFE membrane (b), and PA-PTFE membrane (c) for stent covering. Increased smoothness of stents covered by PA could prevent bacterial adherence and biofilm deposition.
tetrabutydrufuran and then vigorously stirred to obtain a homogenous solution. PTFE-covered self-expandable metal stents were fixed on a Teflon bar (ø: 10 mm), dipped into PA solution, withdrawn, and air dried. Gem-PA-PTFE stents were dried at room temperature for five days and then used for further studies.

2.4. Field emission-scanning electron microscopy (FE-SEM)

Surface morphology of PA-PTFE and PTFE was observed with FE-SEM (Hitachi S-4800, Tokyo, Japan). Stent membranes were sliced into small pieces, mounted, sputter coated with gold using an ion coater, and then observed at an accelerating voltage of 20 kV.

2.5. Water contact angle measurements

Static water contact angles on the film surfaces were characterized using the sessile drop method to characterize membrane properties. The contact angles of water onto PTFE and PA-PTFE were measured at room temperature using a contact angle meter (Phoenix 150, SEO Contact Angle Analyzer, SEO Co., USA) equipped with a special optical system and a charge-coupled device camera. A drop of liquid (10 μL) was placed with 1 μL/s velocity on film and contact angles were measured after 5 s. Measurements were performed at five different points on each film and the mean value was calculated.

2.6. In vitro release study

Gem-PA-PTFE stents were placed inside 15 mL conical tubes, and 10 mL of 0.01 M phosphate buffered saline (PBS) was added. Release studies were performed in a shaking water bath at 37 °C and 50 rpm. The PBS in each tube was collected and replaced at specified times, and the concentration of GEM in the buffer was determined with an ultraviolet (UV) detector at 266 nm.

2.7. Cell culture

A CT-26 murine colorectal carcinoma cell line was purchased from Korea Cell Line Bank (Seoul, Korea) and maintained in Dulbecco’s modified Eagle’s medium (DMEM) (WelGENE Biopharmaceuticals, Daegu, Korea) containing 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37°C for 5% CO₂.

2.8. Apoptotic activity of Gem during 30-day release study

The biological stability (apoptotic activity) of Gem during the release study was evaluated by annexin V staining. Released Gem was periodically recovered during 30 days, diluted, and introduced to CT-26 cells. The treatment concentration was adjusted to the IC₅₀ of Gem, which was pre-determined by MTT assay (Fig. S4). Cells were treated for 24 h, washed with media, and incubated for another 48 h. Apoptosis was determined using the ApoAert™ Annexin V apoptosis kit (Clontech, Palo Alto, CA, USA). In brief, cells were trypsinized and gently washed once with serum-containing media before incubation with annexin V. They were then resuspended in 200 μL of binding buffer, to which 5 μL of annexin V (20 μg/mL in Tris- NaCl buffer) and 10 μL of propidium iodide (50 μg/mL) were added. Cells were incubated for 10–15 min at room temperature in the dark and then analyzed by flow cytometry (Beckman-FC500, Beckman Coulter, USA).

2.9. In vivo inhibition of subcutaneous colon cancer growth

CT-26 metastatic murine colon cells were cultured in DMEM supplemented with 10% FBS. Cells that reached 60–70% confluency were washed with Hank’s balanced salt solution, and detached with trypsin/EDTA, followed by the addition of trypsin neutralizing solution. Cells were resuspended in complete medium for injection. A total of 100 ul of the cell line at a concentration of 1.0 × 10⁶ cells were subcutaneously injected to male BALB/c mice (Orient Co., Korea). When the tumor reached an average diameter of ~6 mm, a Gem-PA-PTFE film was surgically implanted under the tumor. Treatment dose was adjusted to 250 μg of Gem. A total of 20 mice were divided into four groups as follows: 1) non-treated, 2) Gem subcutaneously injected, 3) PA-PTFE film-injected, 4) Gem-PA-PTFE film implanted. Two perpendicular diameters of tumor were measured every two days using Vernier calipers (Mitutoyo Co., Japan), and the volume was calculated using the formula V = (a × b × h)/2, with a being the largest and b being the smallest diameter.

2.10. Histological analysis of Gem-PA-PTFE membrane treated tumors

Tumors were removed five days after film implantation, fixed in 10% formamide, paraffin-embedded, and sliced for hematoxylin-eosin (H&E) staining or a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The TUNEL assay was performed according to the manufacturer’s protocol (Promega Corporation). Briefly, tissue sections were deparaffinized and fixed at room temperature for 5 min in 4% paraformaldehyde. Cells were stripped of protein by incubation for 8–10 min with 20 μg/mL proteinase K. After being rinsed twice with PBS for 5 min, the slides were incubated with terminal deoxynucleotidyl transferase buffer for 10 min. Terminal deoxynucleotidyl transferase and buffer were then added to the tissue sections, which were incubated in a humidified atmosphere at 37 °C for 60 min. The slides were washed three times with PBS for 5 min. The tissues were stained in propidium iodide solution for 15 min at room temperature, mounted on slides, and examined with a fluorescence microscope (Carl Zeiss Microscope System, Jena, Germany). The number of TUNEL-positive cells was counted in six randomly selected fields (20×) and the apoptosis index for each field was calculated as the percent of TUNEL-positive cells relative to the total number of cells.

2.11. Organ distribution of Gem

CT-26 colon tumor-bearing BALB/c mice were treated with Gem-PA-PTFE film in the same manner as the tumor growth study. Organs and blood were recovered at 1, 5, 10, and 20 days after implantation and the concentrations of Gem estimated. Organ tissues were homogenized after dilution with PBS and 400 μL of blood was collected by cardiac puncture into EDTA-containing tubes and centrifuged at 3000 × g and 4 °C for 15 min. Aliquots of plasma were mixed with 50 μL of 5-sulfosalicylic acid

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**Table 1**

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Degree of acetylation</th>
<th>Coating thickness (μm)</th>
<th>Contact angle (unit:°)</th>
<th>GEM concentration per unit area (μg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA-PTFE I</td>
<td>1.18</td>
<td>37.7 (±4.8)</td>
<td>53.6 (±1.2)</td>
<td>186.05 (±11.2)</td>
</tr>
<tr>
<td>PA-PTFE II</td>
<td>1.71</td>
<td>42.7 (±1.3)</td>
<td>57.9 (±1.8)</td>
<td>212.7 (±49.8)</td>
</tr>
<tr>
<td>PA-PTFE III</td>
<td>2.10</td>
<td>52.3 (±6.2)</td>
<td>62.8 (±1.8)</td>
<td>216.5 (±60.6)</td>
</tr>
</tbody>
</table>

* a Number of acetyl groups per one glucose unit of pullulan, estimated by NMR.
  b Measured by micro-meter.
  c The contact angle of PTFE film is 103.9° ±3.0°.
  d GEM concentration was analyzed by UV–Vis spectroscopy at 266 nm.

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**Fig. 2.** Sustained release of Gem from PA-PTFE films. PA with a higher degree of acetylation showed more sustained release of Gem (n = 3).
solution (10%, w/v) on ice, centrifuged to get clear supernatants, filtered, and evaluated by HPLC (2695 system, Waters, USA). Levels of Gem and its metabolite (dFdU) were determined by a validated HPLC method. Chromatographic separation was carried out at room temperature using a Thermo Scientific Hypersil C18 column (150 × 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 and its metabolite was detected at 269 nm. The inserted films were recovered and the residual amount of Gem in the films was determined by HPLC.

3. Results and discussion

3.1. Physicochemical characteristics of PA-PTFE membrane stent cover

According to our non-vascular DES design, PTFE was employed as the prime layer, and PA was designated as the drug-loading controlled-release matrix (Fig. 1a). Table 1 shows that the thickness of the PA-PTFE membrane increased from 37.7 to 52.3 μm with the degree of acetylation (Table 1). Gem loading in the membrane was also increased by the degree of acetylation, but not significantly affected. Overall Gem loading was approximately 186.1–216.5 μg/cm².

Measurement of water contact angles showed the hydrophilicity of the membrane was increased after laying PA on the PTFE (Table 1). The water contact angles of PA-PTFE membranes were 53.60 ± 1.17°, 57.94 ± 1.82°, and 62.81 ± 1.78° for PA-PTFE I, PA-PTFE II, and PA-PTFE III, respectively, and increased by the acetylation of PA. The water contact angle of PTFE, which is known as a typical hydrophobic material, was 103.93 ± 3.01°. These results suggested that PA increases the hydrophilicity of the membrane and possibly increases the tissue compatibility. SEM observation of the membrane demonstrated that the surface was smoother after PA coating without any cracking or webbing (Fig. 1b and c). Generally, hydrophilicity and membrane smoothness influence thrombus formation and neo-intimal hyperplasia. The increase of hydrophilicity and smoothness

Fig. 3. Long term biological stability of the released Gem from Gem-PA-PTFE stent membranes. The Gem released from the stent membranes for 30 days did not show any lose of apoptotic activity which was confirmed by annexin V binding efficiency.
found with the introduction of PA appear to be promising attributes which should be beneficial in the development of a stent cover.

3.2. In vitro release of Gem

The Gem release behaviors of PA-PTFE membranes under simulated physiological conditions (PBS, pH 7.4, 37 °C) were investigated and compared (Fig. 2). The initial burst of Gem, which was calculated based on the % released within 6 h, was 50% (released amount: 93.0 μg/cm²) from PA-PTFE I, 30% (63.8 μg/cm²) from PA-PTFE II, and 15.1% (32.7 μg/cm²) from PA-PTFE III. Gem-loaded polyurethane-PTFE (Gem-PU-PTFE) prepared by the same method showed an initial burst of Gem > 80% on day 1 of the study and release was not sustained for more than a month (Fig. S3). PA-PTFE III, with the highest degree of acetylation, showed the greatest sustained release of Gem and was thus applied in subsequent experiments. The sustained release of Gem from PA-PTFE membrane seemed from the formation of hydrogen bonding between OH-groups of gemcitabine and acyl groups of acetylated pullulan [32]. As a result of the release properties associated with Gem-PA-PTFE, toxic side effects caused by high release of drug due to initial bursts appears to be effectively inhibited which is important to ensure that patients receive safe and appropriate drug doses.

3.3. In vitro antitumor efficacy of Gem-PA-PTFE membrane

Based on the release study, we assumed Gem would be biologically active at least for 30 days; therefore, biological stability (apoptotic activity) of the released Gem from the PA-PTFE films for 30 days was evaluated by annexin V staining. Fig. 3 shows that the apoptotic activity of the released Gem was maintained even after 30 days of incubation. The fraction of Gem released at 30 days induced 54% apoptosis and 10% secondary necrosis from the total treated CT-26 cells. These values were identical to those of the initially released Gem. These data demonstrate that Gem-PA-PTFE films are capable of prolonged drug release and antitumor cytotoxicity in vitro throughout the 30-day elution period without any lose of biologic activity.

3.4. In vivo efficacy of Gem-PA-PTFE film on tumor growth

Several studies have demonstrated the ability of drug-eluting polymers to arrest tumor growth [33,34]. The present study, using Gem-PA-PTFE films, further establishes that polymeric films are capable of providing prolonged low-dose delivery of a chemotherapeutic agent to prevent tumor growth when compared to an equivalent dose of Gem delivered systemically. It is likely that local delivery of anti-proliferative, anti-migratory, anti-thrombotic, and anti-inflammatory drugs can prevent stent restenosis [2,35]. The in vivo antitumor activity of Gem-PA-PTFE film against CT-26 murine colorectal tumors is summarized in Figs. 4 and 5. The tumors in the control group mice grew almost exponentially, but those in the Gem-PA-PTFE film-inserted group were significantly inhibited. Remarkably, tumors rapidly diminished in response to the Gem-PA-PTFE films, accompanied by massive apoptosis, and completely disappeared by the seventh day of treatment. The treatment of free Gem (0.5 mg/mice) at the same dose as that in the Gem-PA-PTFE films

Fig. 4. In vivo inhibition of tumor growth. Stent membranes (Gem-PA-PTFE and PA-PTFE) and Gem solution were inserted or injected at subcutaneous sites. Gem-eluting stent membranes (Gem-PA-PTFE) induced the total regression of subcutaneous CT-26 tumors (D).
showed no in vivo anti-cancer effect and no significant difference compared to the non-treated group. The average tumor volumes at day 14 after initiation of therapy were 1402.5 mm$^3$ for the non-treated group, 1084.4 mm$^3$ for the PA film-inserted group, 1222.8 mm$^3$ for the Gem-injected group, and 0 mm$^3$ for the Gem-PA-PTFE film-inserted group (Fig. 5a). All animals were euthanized on day 14. Fig. 4 shows representative photographs of each group's tumors after the experiment. The tumor from the Gem-PA-PTFE film-implanted mouse is the smallest among those tested. We also compared body weights of the groups over time to evaluate the systemic toxicity of Gem-PA-PTFE films. No serious adverse effects were observed as measured by weight loss, ruffling of fur, behavior, or feeding during the experimental period (Fig. 5b); therefore, Gem-PA-PTFE film was documented as not inducing systemic toxicity. Taken together, these data suggest that the anti-cancer effect of Gem was significantly enhanced by incorporating it into PA film. A previous article reported that Gem exerts a cytotoxic effect against cancer cell in both dose- and time- dependent manners [36]. The increase of Gem exposure time from 2 to 24 h resulted in an increase in cytotoxicity up to 75-fold of the IC$50$, which indicates that the duration of Gem exposure appears to be an important parameter with regards to its anti-cancer effect [37]. Gem-PA-PTFE film continuously released Gem, which enabled prolonged contact of Gem with the tumors, resulting in effective exertion of its anti-cancer effects.

3.5. Histological observation of Gem-PA-PTFE treated tumors

Gem incorporates into DNA during S phase and induces apoptosis [38]. Apoptosis is also an active process of cancer cell death that occurs in response to a variety of agents including anticancer drugs. We confirmed this induced apoptosis using H&E staining and a TUNEL assay five days after subcutaneous tumor implantation (Fig. 6a). Small numbers of apoptotic cells were detected in tumors treated with free Gem and PA film-inserts (without drug), whereas tumor cell apoptosis was 10 times greater following Gem-PA-PTFE film treatment (Fig. 6b). TUNEL staining showed that free Gem alone induced 3.24% apoptosis, while Gem-PA-PTFE film induced 32.2% apoptosis in the tumor tissue, indicating that Gem-PA-PTFE film is highly effective. Adjacent sections were stained with H&E and high levels of condensed apoptotic nuclei, another apoptotic feature, together with necrotic tumor cells were observed in tumors from Gem-PA-PTFE film-treated animals.

3.6. Biodistribution of Gem

We examined the effect of Gem on the viability of colon cancer cells. Cells were exposed for 24 h with varied concentrations of Gem (0.05 μg·mL$^{-1}$ - 5 μg·mL$^{-1}$) and evaluated after 72 h by MTT assay. The IC$50$, defined as the concentration of Gem inducing 50% growth inhibition, was 0.13 μg·mL$^{-1}$ (Fig. S4). This value suggested the minimum local concentration to be maintained for long term (<30 days, as shown in a previous dissolution study) delivery should be >0.13 μg·mL$^{-1}$. Based on the release study, it was assumed that Gem-PA-PTFE stents could maintain the required concentration at the tumor site for an extended period. Tumor concentrations of Gem with Gem-PA-PTFE treatment were verified to confirm that assumption. Fig. 7 shows that Gem accumulated in tumors in higher concentration than in any other organs (i.e., 16.4, 5.94, 5.80 x > blood, muscle, and liver). After 10 days of treatment, the tumors totally regressed and the level of Gem could no longer be estimated in the tumors. However, Gem concentration in other organs maintained the same levels as those determined at days 1 and 5. We assumed that the Gem-PA-PTFE membrane worked as a local delivery system without systemic exposure of Gem. We discovered that Gem remained on PA film for more than 20 days of treatment as was observed in the previous release study (Fig. S5). Localization of Gem inside tumors with extremely low systemic exposure could prevent systemic side effects. Intravenous injection or infusion of cancer drugs easily attains high plasma concentrations; however, previous pharmaco kinetic studies of chemotherapeutic agents have illustrated that both diffusion into and clearance from tumors is significantly delayed, resulting in subtherapeutic drug levels within tumors [39,40]. In addition, as demonstrated in the current study, rapid plasma clearance prevents significant accumulation of systemically administered Gem in the local tumor of interest, thus permitting tumors to grow unabated. Gem-PA-PTFE films take advantage of several polymer-based properties to deliver carrier-free Gem locally over several weeks via specifically engineered polymer films which thereby improves local bioavailability, significantly increases drug tumor levels, and avoids the early metabolization of the active compound which can be associated with a single systemic dose of Gem.
4. Conclusion

In this study, we investigated the capacity of PA-PTFE membrane as a non-vascular metal stent cover to control drug release of the hydrophilic cancer drug Gem. The release of Gem from PA-PTFE stents was sustained for 30 days. Subcutaneous CT-26 colon tumors totally regressed following treatment with Gem-PA-PTFE film without systemic exposure of Gem or its related toxicities. The

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**Fig. 6.** PA-GEM films showed (a) H&E staining of tumor and significant enhancement of apoptosis (b) quantified by counting the number of TUNEL-positive cells in microscopic findings from BALB/c mice bearing CT-26 tumors. *P* value was calculated with Mann Whitney and *t*-tests (***p < 0.006***).

**Fig. 7.** Tissue distribution of Gem eluted from Gem-PA-PTFE membrane (a). Local and sustained release of Gem from the membrane resulted in a higher Gem concentration in local tumor tissues (*n* = 4). The residual amount (%) of Gem in the Gem-PA-PTFE stent membranes (b). *Gem concentrations at tumor were not measured from total regression of tumor.*
successful controlled release of Gem from PA-PTFE covered DES increases the potential usefulness of DES for the treatment of malignant gastrointestinal cancer as well as cancer-related stenosis.

Acknowledgements

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Appendix

Figures with essential colour discrimination. Certain figures in this article, particularly Figs. 1, 3, 4, 6 and 7 are difficult to interpret in black and white. The full colour images can be found in the online version, at doi:10.1016/j.biomaterials.2011.01.070.

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biomaterials.2011.01.070.

References