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In-vitro elution of cisplatin and fluorouracil from bi-layered biodegradable beads

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Abstract. This study developed biodegradable bi-layered drug-eluting beads and investigated the in-vitro release of fluorouracil and cisplatin from the beads. To manufacture the drug-eluting beads, poly[(d,l)-lactide-co-glycolide] (PLGA) with lactide:glycolide ratios of 50:50 and 75:25 were mixed with fluorouracil or cisplatin. The mixture was compressed and sintered at 55℃ to form bi-layered beads. An elution method was employed to characterize the release characteristic of the pharmaceuticals over a 30-day period at 37℃. The influence of polymer type (i.e., 50:50 or 75:25 PLGA) and layer layout on the release characteristics was investigated. The experiment suggested that biodegradable beads released high concentrations of fluorouracil and cisplatin for more than 30 days. The 75:25 PLGA released the pharmaceuticals at a slower rate than the 50:50 PLGA. In addition, the bi-layered structure reduced the release rate of drugs from the core layer of the beads. By adopting the compression sintering technique, we will be able to manufacture biodegradable beads for long-term drug delivery of various anti-cancer pharmaceuticals.

Keywords: biodegradable bi-layered beads; polylactide-polyglycolide (PLGA); in vitro release; fluorouracil; cisplatin

1. Introduction

Presently the most curative treatment option for solid tumors is surgical resection followed by adjuvant chemotherapy or radiation therapy to minimize the risk of recurrence. However, intravenously administered chemotherapy for these tumors has limited effectiveness. Since only a small amount of the systemic blood flow is directed to the tumor, only a fraction of the total dose reaches the tumor site (Anand *et al*. 2000, Conchita *et al*. 2010) The remainder of the dose is distributed throughout healthy organs and tissues, leading to a variety of undesirable side effects ranging from neutropenia to cardiomyopathy (Crawford *et al*. 2004, Wallace 2003). Many chemotherapeutic drugs also have very rapid plasma clearance, leading to short tumor exposure times (El-Kareh and Secomb 2000, Di *et al*. 2013) To improve the outcome of these cancer patients, a new paradigm of minimally invasive and locoregional cancer therapies has rapidly

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evolved and received considerable attention in the recent years (Gillams 2005, Bai and Liu 2014). Administering an anti-cancer drug either to the region that contains a tumor or directly within the tumor has the advantage of increasing tumor exposure to a drug while limiting systemic toxicity (Blanco *et al.* 2008).

Cisplatin and fluorouracil (5-FU) have been widely used to treat cancers of various origins, for example, esophagus, gastrointestinal tract, urinary bladder, and head and neck. Cisplatin is a platinum-containing compound. The platinum complexes react with cellular DNA, forming both intrastrand and interstrand cross-links, inhibiting DNA replication and transcription, and leading to breaks and miscoding. 5-FU belongs to the family of pyrimidine analogs. 5-FU requires intracellular enzymatic conversion to nucleotide in order to exert its cytotoxic activity. One of the active metabolites, 5-fluoro-2´-deoxyuridine-5´-monophosphate (F-dUMP), is a potent inhibitor of thymidylate synthase, an enzyme necessary for the synthesis of deoxythymidine triphosphate (dTTP) and ultimately DNA. Other actions of 5-FU nucleotides include incorporation into DNA and inhibition of RNA processing. When used in combination, cisplatin can act as a modulator of the cytotoxic activity of 5-FU, leading to a higher tumor response rate (Blanco *et al.* 2008). These two drugs are available for intravenous dosing. However, potential side effects associated with the systematic use of cisplatin and 5-FU for chemotherapy include nausea or vomiting, immunosuppression, myelosupression, sore mouth, constipation, diarrhea, numbness or tingling in hands or feet, fatigue, and an inflammation of the lining of conjunctiva (Peter and Van Groeningen 1991, Einhorn 1990). An ideal drug delivery system for chemotherapy should: 1) provide adequate pharmaceutical concentration at the target site, 2) maintain a slow and constant release of drugs over a prolonged period, and 3) be biodegradable so that a second operation for its removal is not required. Biodegradable beads made of anti-cancer drugs may provide an ideal alternative for the treatment of patients with carcinomas.

Biodegradable beads made out of polymers incorporating anti-tumor drugs have advantages in four ways. First, biodegradable beads provide high concentrations of anti-cancer pharmaceuticals at the target site for a prolonged time needed to completely treat the particular carcinoma. Second, variable biodegradability from weeks to months may allow many types of cancers to be treated. Third, because the biodegradable beads dissolve, there is no need for surgical removal; and fourth, because the biodegradable beads dissolve slowly, allowing time for tissue ingrowth, there is no need for reconstruction.

This current paper explores the alternative of processing biodegradable polymers as bi-layered beads that provide a controlled sustained release of cisplatin and 5-FU. Polylactide-polyglycolide (PLGA) copolymers with two different lactide:glycolide ratios, namely 50:50 and 75:25, were employed. As far as the authors can know, there is no other research that proposed such biodegradable beads providing sustainable releases of two anti-cancer drugs. We adopted a compression-sintering technique (Harkin *et al.* 2003, Chan *et al.* 2007, Sanjeeb and Sarbari 2011, Alanazi1 *et al.* 2014, Chung *et al.* 2010) to manufacture bi-layered polymer/cisplatin/5-FU beads of various sizes. Beads were evaluated by an in-vitro elution method and an HPLC assay. The effect of different process parameters on the release characteristics of cisplatin and 5-FU was investigated. In addition, scanning electron microscopy was also employed to observe the surface morphologies of the biodegradable beads.

2. Experimental procedure

2.1 Polymeric materials

Bi-layered beads that incorporate anti-cancer pharmaceuticals were fabricated in this study. Two types of polylactide-polyglycolide (PLGA) were used including a lactide:glycolide ratio of 50:50 (Resomer RG 503, Sigma-Aldrich, USA) with a molecular weight of 33,000 Da and a lactide:glycolide ratio of 75:25 (Resomer RG 756, Sigma-Aldrich, USA) with a molecular weight of 70,000 Da. The molecular weights of the polymers were measured by a Gel Permeation Chromatograph equipped with a Waters 2414 Refractive Index Detector. All polymers were commercially available from Sigma-Aldrich (Saint Louis, MO, USA) and were in powder form with particle size ranges from 100 to 200 m. A DuPont model TA-2000 differential scanning calorimeter was used to characterize the thermal properties of the polymers. The measured results suggested that all polymers' glass transition temperatures were in the range of 40-55℃. The anti-cancer drugs used included cisplatin and 5-FU powder with a particle size of 100-200 m, also purchased from Sigma-Aldrich (Saint Louis, MO, USA).

2.2 Fabrication of bi-layered beads

The polymer/cisplatin and polymer/5-FU were separately pre-mixed by a lab scale dry mixer. The mixture was compressed into beads of different diameters (3 and 5 mm) by a mold shown schematically in Fig. 1. The compressed beads with the mold were then placed in an oven for sintering. The sintering temperature was set at 65℃, which was higher than polymers' melting point, but low enough to avoid destroying the pharmaceuticals. The sintering time used was 30 minutes in order to attain an isothermal sintering of the beads. Beads of 3 mm in diameter were first manufactured. They were then covered with another layer of polymer/drug to form bi-layered beads with an outside diameter of 5 mm by the same compression sintering method. Table 1 lists the contents of the polymers and pharmaceuticals used in the experiments.

Fig. 1 Photo of the mold used to fabricate the 5 mm beads

Table 1 Composition of bi-layered beads

Run	Core (external diameter=3 mm)		Surface layer (internal diameter=3 mm, external diameter=5 mm)	
	Drug (4 mg)	Polymer type (LA:GA) (20 mg)	Drug (16 mg)	Polymer type (LA:GA) (80 mg)
A	5-FU	75:25	Cisplatin	50:50
B	5-FU	50:50	Cisplatin	75:25
C	Cisplatin	75:25	5-FU	50:50
D	Cisplatin	50:50	5-FU	75:25

2.3 Characterization of drugs release

An in-vitro elution method was employed to determine the release characteristics of cisplatin and 5-FU from the bi-layered biodegradable beads. A phosphate buffer, 0.15 mol/L (pH 7.4), was used as the dissolution medium. Beads were placed in glass test tubes with a volume of 1 ml phosphate buffer. All tubes were incubated at 37℃. The dissolution medium was collected and analyzed at every 24 hour interval. Fresh phosphate buffer (1 ml) was then added for the next 24 hour period and this procedure was repeated for 30 days. The drug concentrations in buffer for the elution studies were determined by a high-performance liquid chromatography (HPLC) assay standard curve for cisplatin and 5-FU. The HPLC analyses were conducted on a Hitachi D-2000 Elite Delivery System. The column used for separation of both drugs was a ZORBAX ODS, C18, 5 m, 4.6 cm×250 mm HPLC column. The mobile phase used for the characterization of cisplatin contained acetonitrile (Sigma-Aldrich, USA) and distilled water (90/10, v/v). The absorbency was monitored at 300 nm and the flow rate was 2.0 ml/min. On the other hand, the mobile phase used for 5-FU was composed of methanol (Sigma-Aldrich, USA) and distilled water (10/90, v/v), while the absorbency was monitored at 265 nm and the flow rate was 1.0 ml/min. All samples were assayed in triplicate and sample dilutions were performed to bring the unknown concentrations into the range of the assay standard curve. A calibration curve was made for each set of the measurements (correlation coefficient>0.99). The eluted drugs can be specifically identified and quantified with high sensitivity using HPLC system.

3. Results

By employing the compression-sintering method, bi-layered biodegradable beads could be successfully fabricated. Fig. 2 shows photographically the manufactured beads.

The drug concentrations in buffer for the elution studies were determined by a high-performance liquid chromatography (HPLC) assay. The HPLC assay results for cisplatin and 5-FU standard curves with five different standard concentrations (1, 10, 100, 500 and 1000 g/ml) are showed in Figs. 3(a) and 3(b) respectively. The calibration fittings for these curves are

$$[\text{Cisplatin}] \qquad \text{Peak area} = 121.35 \times \text{concentration} - 952.27 \quad R^2 = 0.9975 \qquad (1)$$

$$[5\text{-}FU] \qquad \text{Peak area} = 22571 \times \text{concentrat} - 82631 \qquad R^2 = 0.9975 \qquad (2)$$

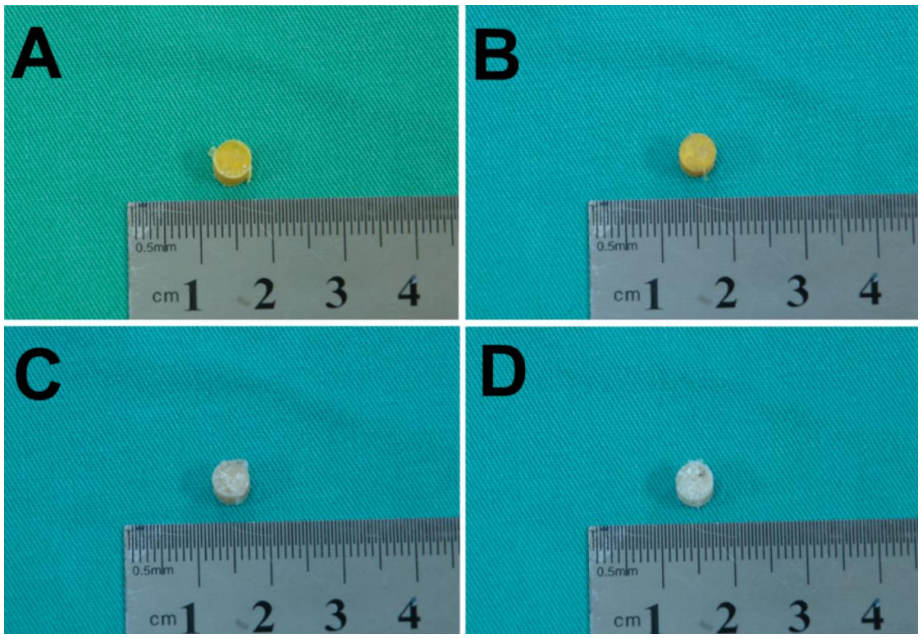
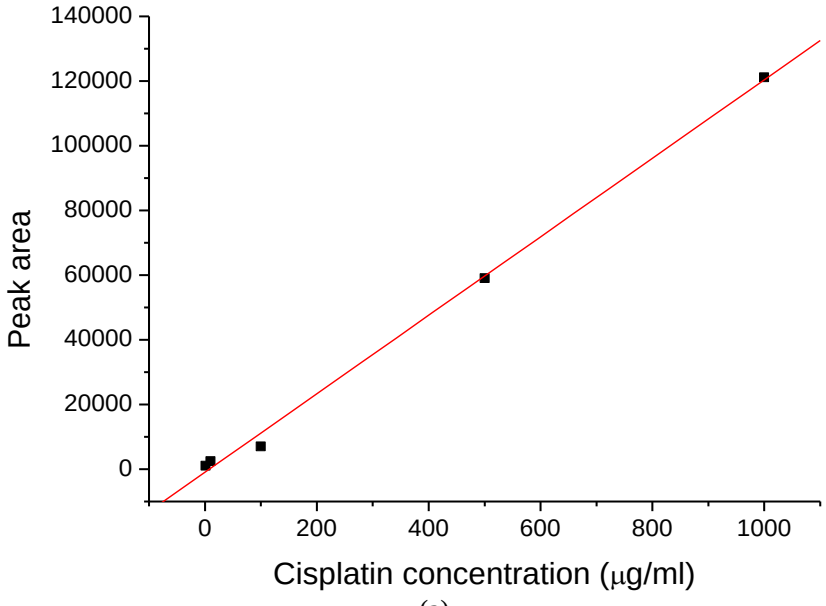
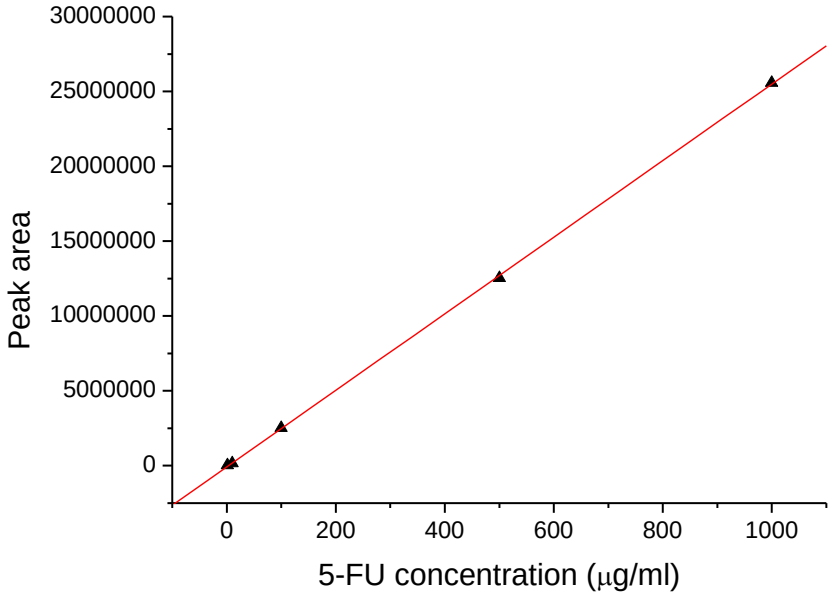


Fig. 2 Photos of the fabricated beads (figures A, B, C, and D correspond to the processing conditions of A, B, C, and D respectively in Table 1)



(a)

Fig. 3 Continued



(b)

Fig. 3 HPLC standard curves for (a) cisplatin and (b) 5-FU.

3.1 Release characteristics of bi-layered beads: cisplatin as the surface layer

The in vitro release curves of cisplatin and 5-FU from the bi-layered biodegradable beads are shown in Figs. 4 and 5 respectively. All curves exhibited an initially burst release during the first five days, followed by a more gradual and sustained release of the drugs until day 15, after which a second peak in release was observed at day 20. In addition, the experimental results in Figs. 4 and 5 suggest that biodegradable beads could release high concentrations of cisplatin and 5-FU for more than 30 days.

When PLGA/cisplatin was used as the surface layer while PLGA/5-FU was used as the core, the beads exhibited more severe burst release of cisplatin (Fig. 4). Beads with 50:50 PLGA as the matrix material for surface layers showed higher initial cisplatin release than those with 75:25 PLGA as the surface matrix material. This might be due to the fact that 50:50 PLGA has a less molecular weight and degrades faster than 75:25 PLGA. The incorporated cisplatin in the 50:50 PLGA surface layer thus released faster and show a higher burst release. Furthermore, when cisplatin was used and embedded in the surface layer, due to a high drug loading (80 mg), the beads released a higher drug concentration of cisplatin than that when the drug was used as the core layer. On the other hand, due to the barrier provided by the surface layer, the 5-FU incorporated at the core of the beads showed a much lower initial drug release characteristic. Again the beads with 50:50 PLGA as the matrix material for surface layers showed higher initial release of 5-FU than those with 75:25 PLGA as the surface matrix material. In addition, despite the drug release of 5-FU was much lower than that of cisplatin, mainly due to the lower total 5-FU loading of the beads (20 mg), the concentration of 5-FU increased significantly after 15 days and were comparable to that of cisplatin at days 20-25.

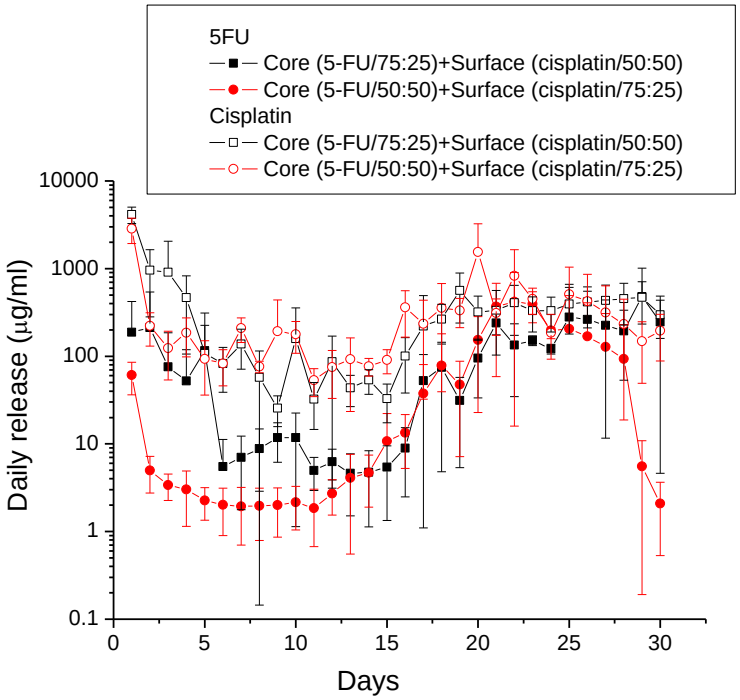


Fig. 4 Release curves of cisplatin and 5-FU from biodegradable bi-layered beads with 5-FU incorporated at the core layer and cisplatin distributed at the surface layer

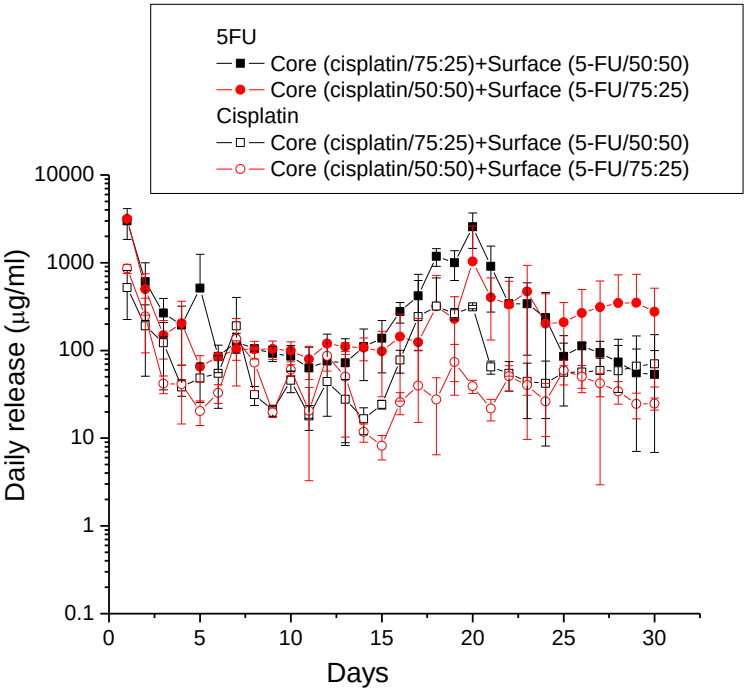


Fig. 5 Release curves of cisplatin and 5-FU from biodegradable bi-layered beads with cisplatin at the core layer and 5-FU at the surface layer

3.2 Release behaviors of bi-layered beads: 5-FU as the surface layer

When 5-FU was distributed at the surface while cisplatin was embedded at the core of the beads, the burst release of cisplatin was reduced (Fig. 5). The total release rate of cisplatin was reduced as well. This is attributed to the fact that the surface PLGA/5-FU layer acts a barrier for cisplatin to be eluted from the beads. The release rate of cisplatin decreased accordingly. Generally the beads with 50:50 PLGA as the matrix material for surface layers showed higher initial release of cisplatin than those with 75:25 PLGA as the surface matrix material. Again this can be explained by the fact that the 50:50 PLGA provides less shielding effect for the drugs at the core. Release cisplatin increased accordingly.

As expected, the beads showed a higher release of 5-FU when 5-FU was located at the surface layer. This is due to that the total drug loading of 5-FU (80 mg) at the surface layer is higher than that of cisplatin (20 mg) at the core. In addition, no barrier was provided for 5-FU distributed at the surface layer. Beads thus released higher concentrations of 5-FU during the elution process. In addition, the beads released higher drug concentrations of 5-FU than cisplatin for the elution period (i.e., 30 days). Interestingly, the polymer type (either 50:50 or 75:25) did not show significant influence on the release behavior of 5-FU, mainly due to the fact that 5-FU is distributed at the surface layer of the beads.

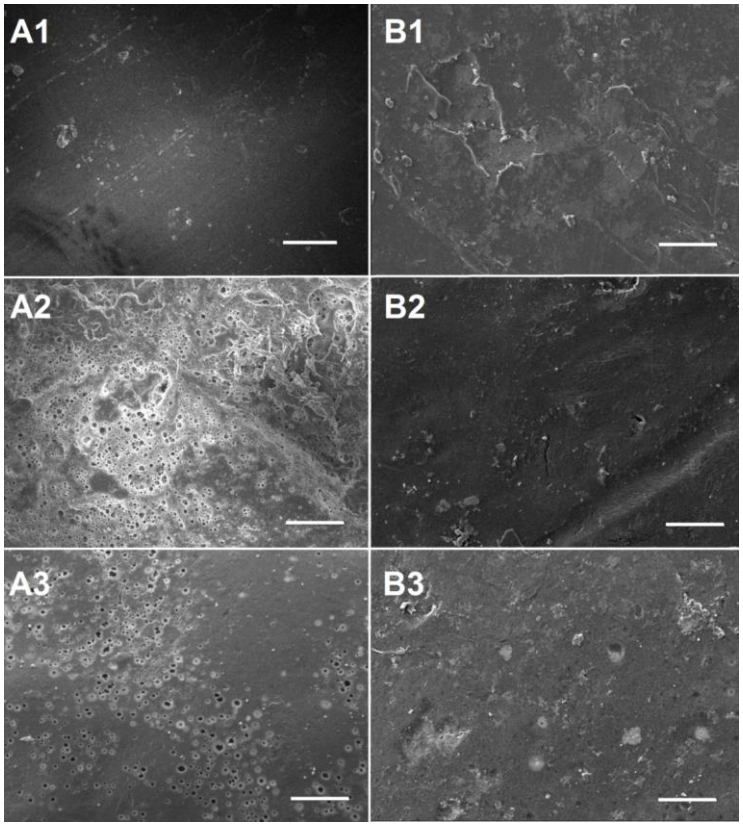


Fig. 5 SEM photos of biodegradable beads right at day 0 (A1, B1), 3 (A2, B2) and 10 (A3, B3) days of elution in the PBS (A: cisplatin at the surface layer, B: 5-FU at the surface layer)

3.3 Surface morphology of eluted beads

Fig. 6 shows the results of the in vitro degradation analysis. The gross appearance of the drug-eluting beads show similar changes as noted on the SEM photographs. At day 0, the beads essential exhibited smooth surfaces, although some scratch marks could be observed (Figs. 6A1 and 6B1) on the surfaces. Three days after the elution, tiny pores on the surface of the implants could be observed, which indicated channel diffusion of the drug from the implants. Bi-layered beads with cisplatin as the surface layer (Fig. 6A2) exhibited more obvious porous structure than the beads with 5-FU at the surface layer (Fig. 6B2). After ten days of elution, due to the osmotic pressure of the solution as well as degradation of the polymeric materials, the pores on the cisplatin surface reduced somewhat (Fig. 6A3) while the pores of 5-FU surface increased and exhibited rough surface (Fig. 6B3).

4. Discussion

Cancer remains a deadly threat despite the best efforts of clinical science. Biodegradable materials are not only being used as implants for temporary skeletal repairs, they can also be used to administer anti-cancer drugs. The release profile should have an initial high release rate to kill cancer cells, followed by 4 to 6 weeks of a relatively constant release above the effective concentrations doing the least harm possible to healthy cells. Various types of polymeric materials have been employed based on their ability to achieve a sustained release of pharmaceuticals. Polylactide-polyglycolide (PLGA) is one of the most promising biodegradable bio-materials (Kau *et al.* 2010, Middleton and Tipton 2000). It is non-toxic and induces minimal inflammatory response, and can be eventually absorbed without any accumulation in the vital organs. There are several anti-cancer pharmaceuticals available for the use of drug-eluting beads. However, specific characteristics should be considered prior to the therapeutic selection: the drugs should be water soluble, anti-cancer cell proliferation, non-toxic to tissue, readily available in powder form, heat stable, and have a low rate of allergic reaction. Both cisplatin and fluorouracil (5-FU) fit these requirements and have been widely employed in antineoplastic treatments.

The release kinetics of drugs from the biodegradable beads was not only found to be influenced by the type of polymer utilized, but also the structure of the beads. To control the release of pharmaceuticals from the biodegradable PLGA devices, various techniques may be used including: First, lactic acid may be copolymerized with glycolid acid. The degradation rate can be varied with the percentage of glycolic acid in the copolymer-the higher the ratio of the lactic acid the longer dissolution time for the poly (DL-lacitde-co-glycolide) beads. Second, the rate of diffusion across PLGA beads can be adjusted by varying the molecular weight of the PLGA-the lower the molecular weight, the faster the degradation rate. By adopting a higher molecular weight or a higher LA ratio polymer in the experiments, the release rate of the beads can be slowed.

During the compression-sintering of polymer beads, the formation of a homogeneous melt from powder particles involves two steps: First, the polymeric particles stick or fuse together at their points of contact around the drug particles. This fusion zone grows until the mass becomes a three-dimensional network, with relatively little density change. Second, at some point in the fusion process, the network begins to collapse into the void spaces between the polymer and the drugs. These spaces are filled with molten polymer that is drawn into the region by capillary forces. The pharmaceutical particles are then encapsulated by the polymer to form a composite bead. After

sintering, small bubbles may exist due to the encapsulation of air pockets between powder particles of polymers and drugs.

For a water-soluble anti-cancer drug in a hydrophobic PLGA matrix, the release mechanisms are controlled by channel diffusion, osmotic pressure, and polymer degradation [9]. Firstly, when anti-cancer drug loading is low, drug particles will be isolated in the polymer matrix. These particles will not be able to permeate through the polymer at a practically useful rate. With an increase in anti-cancer drug loading, drug particles will connect together to form channels leading to the surface of the bead and be released by channel diffusion (Figs. 6A2 and 6B2), which has a higher release rate at the first few days as shown in Figs. 3 and 4. However, the presence of small air bubbles in the composites may lead to an incompletely encapsulation of the drugs, which accelerates the channel diffusion as well as the drug release. The SEM photos suggest that biodegradable bi-layered beads with cisplatin at the surface layer (Fig. 6A2) exhibited more obviously porous structure than beads with 5-FU at the surface layer (Fig. 6B2). This might explain why the biodegradable beads released high concentrations of cisplatin when the drug is at the surface layer. Secondly, if the polymer matrix surrounding the isolated particles remains intact during the release, the drugs may not be released from these clusters. However, water will be taken up by a water-soluble drug with high osmotic pressure through the polymer, causing swelling of the particle. The polymer matrix may break under this swelling to form openings for drug release (Figs. 6A3 and 6B3). A rather stable release of the drugs was thus observed from day 3 to day 15. Finally, when the polymer molecular weight decreases sufficiently, loss of polymer begins. The drugs will then be released along with this polymer loss. This leads to the second release peaks after day 15 in Figs. 3 and 4.

In this study, it is desirable to be able to adjust the release rate and duration from the anti-cancer beads. Since the anti-cancer pharmaceuticals cannot permeate through the coated copolymer wall, device structure has a significant effect on the release profile. When PLGA/cisplatin was used as the surface layer while PLGA/5-FU was used as the core, the beads exhibited more severe burst release of cisplatin. Nevertheless, due to the barrier provided by the surface layer, the 5-FU incorporated at the core of the beads showed a much lower initial drug release characteristic, as shown in Fig. 4. On the other hand, when 5-FU was distributed at the surface while cisplatin was embedded at the core of the beads, the burst release of cisplatin was again reduced attributed to the shielding effect of the surface layer (Fig. 5). By employing different bead structure, we will be able to control the release rates of various pharmaceuticals. A sequential release of the drugs can also be achieved (Fig. 4).

The anti-tumor effects of the pharmaceuticals incorporated into the biodegradable bead far outweigh any negative inherent effects of the bead itself. A significant advantage of the biodegradable anti-cancer beads is to release a variety of chemotherapeutic agents for the locoregional therapy of cancer. The experimental results in this study suggested that biodegradable beads released high concentrations of fluorouracil and cisplatin for more than 30 days. Furthermore, these bi-layered beads were designed to provide optimal drug release kinetics to improve drug delivery efficiency and antitumor efficacy when treating unresectable tumors. Local pharmaceuticals concentrations can be much greater than the effective drug level for most cancers.

It should be noted that although this study has investigated the in-vitro dissolution rate of the beads, an extended study will be done in the future works to determine the in-vivo release pattern of the beads and whether it is equivalent to the in vitro one.

5. Conclusions

This paper has developed biodegradable bi-layered beads for a long-term anti-cancer drug release. Beads were evaluated by an elution method and an HPLC assay. The effect of different polymeric materials and bead structures on the release characteristics of the pharmaceuticals was investigated. It was found that biodegradable beads released high concentrations of cisplatin and 5-FU and for more than 30 days. The 75:25 PLGA released the pharmaceuticals at a slower rate than the 50:50 PLGA. In addition, the bi-layered structure reduced the release rate of drugs from the core layer of the beads. One can control the release rate and the total effectively release period of cisplatin and 5-FU from the beads by adopting PLGA of different LA:GA ratio and by adopting various beads structures.

Further studies being conducted in our laboratory are investigating the biodegradable anti-cancer beads in animal model, such as the pleural cancer models. Eventually biodegradable beads may be used for the development of custom-tailored anti-cancer drugs to treat various carcinomas.

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