Local Delivery of Doxorubicin for Malignant Glioma by a Biodegradable PLGA Polymer Sheet

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Abstract. *Implantable, biocompatible and biodegradable devices bearing an anticancer drug can provide promising local therapy to patients with malignant disorders. With the aim of treating brain tumors, especially gliomas, a membranous sheet containing doxorubicin was produced by co-polymerization to poly(D,L-lactide-co-glycolide) (PLGA). When release of the drug from the sheet was measured, sustained release continued until day 34. The data contrasted with the burst release from material containing a higher proportion of the drug. In terms of biodegradability, a subcutaneous 3 x 3-mm tetragonal sheet was almost completely absorbed by day 80. When a glioma was implanted subcuteneously and the tumor nodule exposed to the sheet, the device inhibited tumor growth significantly. The sheet consisted of an amorphous structure with cavities estimated to have a diameter of 0.5 – 3 μm by electron microscopic observation. Since the sheet is implantable, biodegradable and has a sustained-drug release property, the device may play a role in the local therapy of brain tumors.*

Malignant brain tumor, such as infiltrating glioma and glioblastoma, is one of the most intractable diseases in the human body. The invasive character and rapid proliferation of the cells often brings recurrence of the disease even after radical treatment and an increase in intracranial hypertension eventually causes herniation due to limited intracranial space. The median survival time is 0.4 years for glioblastoma and is 5.6 years even for more benign low-grade astrocytoma (1). Most patients die within 2 to 5 years after their diagnosis. In spite of recent advances in radiotherapy, immunotherapy, chemotherapy and other adjuvant therapies, the prognosis has not been dramatically improved and more effective therapies are required. Although the prognosis is poor, the tumors seldom metastasize to regions outside of the central nervous system. In addition, the main etiology of death is local recurrence. Therefore, if local recurrence can be prevented, long-term survival or even a complete cure of the patient can be expected.

The main problem of administering chemotherapy for malignancy in the central nervous system is the low efficiency of drug delivery to the residual tumor in brain parenchyma. When anti-malignant drugs are systemically administered, most drugs may not reach the lesion due mainly to the existence of the blood-brain barrier. From the aspect of chemotherapy, alkylating agents such as temozolomide and nitrosourea represented by ACNU or BCNU are the first choice of drugs in combination with radiation (2, 3). These drugs are potent against malignant gliomas since they can cross the blood-brain barrier and enter the tumor cells. They confer toxicity even to not-actively dividing cells, which account for approximately 70% of the brain tumor (4). Moreover, alkylating agents can synchronize cells in the G2M phase and, thus, function as radiosensitizers when combined with therapeutic irradiation. Regardless of such a promising efficacy of the drug, the prognosis of patients has not improved sufficiently. The reason is partly attributable to the low local drug concentration, because the drug delivery is not adequate in spite of penetrability of the drug through the blood-brain barrier (5, 6). When these facts are considered, it is obvious that the development of more potent local treatment is required.

Recent advances in material engineering have provided a new material for such local treatment. One representative example is the BCNU-loaded PLGA wafer (7). PLGA is a biodegradable and biocompatible material, and the BCNU-loaded PLGA wafer is an implantable polymeric device that releases BCNU directly into the tumor tissue. Implanting the device after surgery can eliminate the residual tumor tissue in the operative field and delay recurrence. The antitumor activity of the wafer has been demonstrated (8, 9) and the device might be useful because most patients with glioma undergo surgical removal and chemotherapy as well as radiotherapy.

However, there is a concern about alkylating agent-based local chemotherapy, because tumor cells soon acquire resistance after the systemic administration of drugs. The mechanism of resistance is mainly *via* the recruitment of O_6-methylguanin merthyltransferase, a DNA repair enzyme into tumor cells (10-13). MGMT facilitates stoichiometric transfer of the O_6-alkyl groups from the alkylated DNA molecules to its own cysteine residues and by so doing, is itself deactivated after acceptance of the alkyl groups. Overexpression of MGMT repairs the DNA damage caused by the alkylating agents. Chemotherapeutic agents, such as temozolomide and nitrosourea, induce MGMT expression in the tumor cells and resistance may influence the effect of focal treatment with the BCNU wafer. In such cases, treatment with another anti-malignant drug with a different mechanism of action might be useful. Based on this concept doxorubicin was selected.

The mechanism of doxorubicin resistance is expression of the multiple drug resistant gene (MDR); moreover, it does not show cross-resistance to alkylating agents. In addition, doxorubicin has been used commonly in patients with disseminated lymphoma or leukemia in the cerebrospinal fluid by intrathecal injection and its safety has been well recognized. Thus, doxorubicin was co-polymerized to biodegradable PLGA and a membrane containing the drug was developed. Ultimately, the possibility of modulating the glioma after surgery using the membrane could be explored.

Materials and Methods

Doxorubicin sheet. Doxorubicin hydrochloride ((2S,4S)-4-(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyloxy)-1,2,3,4-tetrahydro-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxynaphthacene-6,11-dione monohydrochloride; DOX or Adriamycin) was provided by Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan). One square centimeter of the sheet contained 1 mg of doxorubicin. To prepare an 8.4 cm^2 surface of the sheet, 8.4 mg of doxorubicin were mixed with 318 mg of PLGA (50:50 molar ratio, Mw53114) dissolved in chloroform. The mixture was co-polymerized by the solvent-evaporation method and used after further desiccation.

Release of doxorubicin in vitro. Measurement of the drug concentration in the solvent was determined by the UV-2200A spectrophotometer (Shimadzu, Kyoto, Japan). The doxorubicin

sheet was set under physiological conditions for days (pH7.4, 37°C in phosphate-buffered saline) and the total amount of the eluted doxorubicin was quantified.

Animal experiments. To investigate the biodegradation of the doxorubicin sheet, closed colony Jcl:ICR mice were purchased from Clea Japan, Tokyo and bred in a standard animal facility. For the tumor implantation and treatment study, five-week old Fischer 344 rats were purchased from Sankyo Laboratory, Tokyo, Japan. These animals were maintained under conditions of 28°C and 55-60% humidity and given free access to food and tap water. All the animal procedures were performed under the guidance of the committee in the animal care facility. In the first set of animal experiments, the 3 x 3 mm tetragon sheet was subcutaneously implanted into the left flank of an ICR mouse. After implantation, absorption of the sheet was determined by weighing the unabsorbed residuals after removal. Degradability was expressed as a percentile of the original sheet weight on the day of observation (n=5 in each group). In the second set of the experiment, the RT2 glioma cell line, syngeneic to the Fischer 344 rat, was used. The RT2 glioma cells were cultured in Dulbecco's minimum essential medium supplemented with 10% bovine serum (GIBCO Laboratories, Grand Island, NY, USA). Three x 10^5 of the trypsinized and dispersed cells in 100 μl of PBS were subcutaneously injected into the rat's right flank and four days later, after confirmation of establishment of the tumor nodule, the rats were treated with 2.1125 cm^2 of doxorubicin sheet containing 2.1 mg of doxorubicin by covering the tumor. For some animals, 8.4 mg/100 μl of doxorubicin were directly injected into the center of the tumor. Tumor volumes were measured and growth was directly accessed. Statistical analysis was performed by the two-tailed Student's *t*-test.

Morphological examination of the doxorubicin sheet by electron microscopy. For the scanning electron microscopy, the doxorubicin sheet was lightly washed in water then fixed with 1.2% glutaraldehyde in 0.1 M phospate-buffered saline then adjusted to pH 7.4. The specimen was dehydrated by ascending concentrations of ethanol and the critical point drying method using liquid CO_2. After the dehydration, the sample was coated with ion-sputtered gold and palladium and observed by a JSM-5800LV Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan) at the accelerating voltage of 15 KV. For transmission electron microscopy, the sheet was fixed with 2% glutaraldehyde in phosphate-buffer and the specimens were subjected to examination by an H-7500 Electron Microscope (Hitachi, Tokyo, Japan) at the accelerating voltage of 100 KV.

Results

Release of doxorubicin from the doxorubicin sheet. The release of doxorubicin from the PLGA membrane was first determined *in vitro*. The concentration of doxorubicin was measured by absorption spectrophotometric analysis. The absorbance of light by doxorubicin in continuous wavelength was measured by a spectrophotometer (Figure 1A) and the correlation of both 232 nm and 480 nm peaks for determination of the doxorubicin concentration was confirmed. The amount of drug in the solvent was
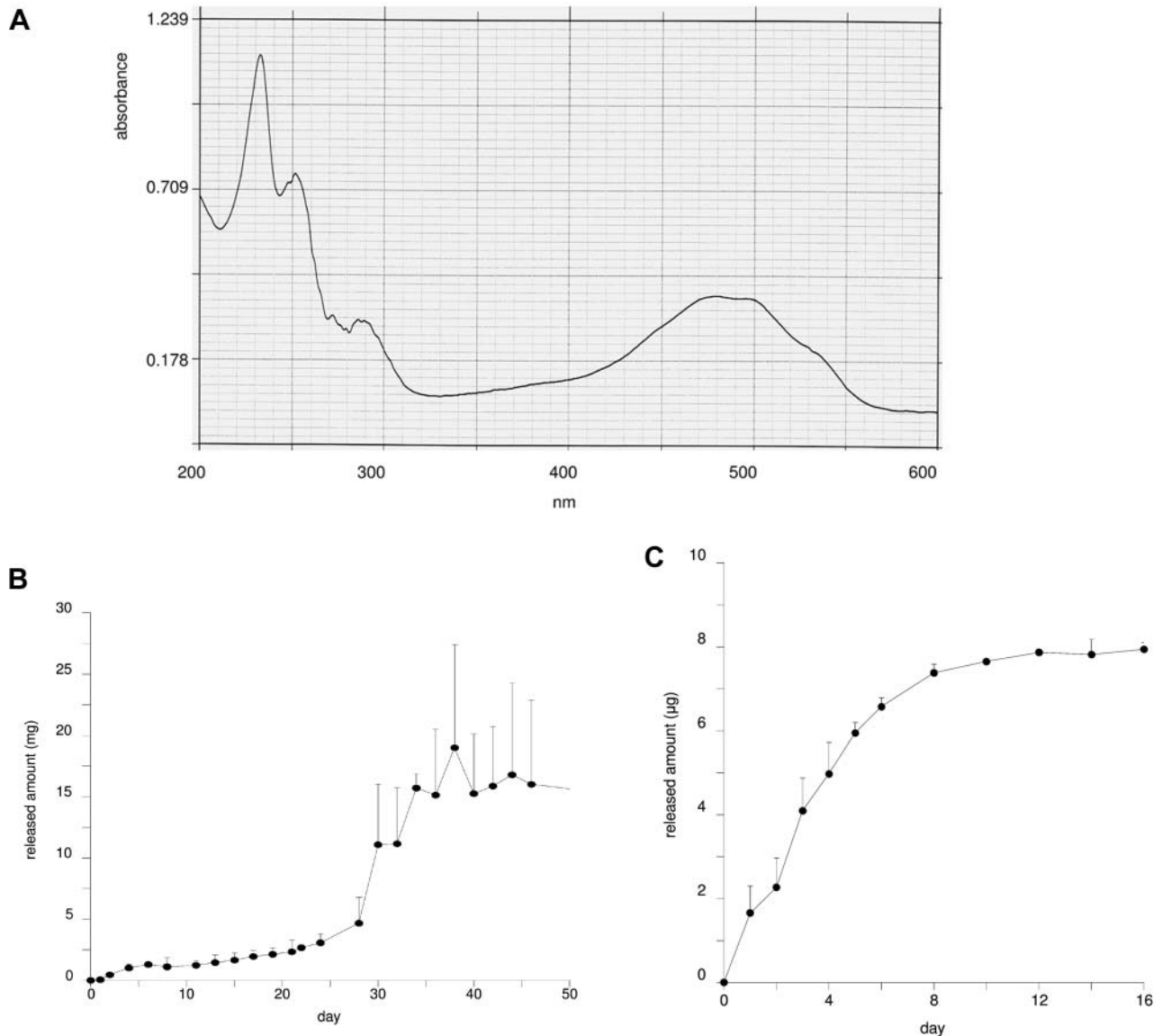
Figure 1. *Release of doxorubicin from the sheet in vitro. A) Spectrophotometric properties of doxorubicin. The light absorbance of doxorubicin was measured by continuous change in the wave length. Based on the figure, the absorbances at 232 nm and 480 nm were used for further determination of the drug concentration. Values measured at both peaks correlated well with drug concentrations. B) Total amount of doxorubicin released from 1 mg of the sheet. Release gradually started from immediately after the exposure and 10% of the drug was released by day 10. The sheet steadily discharged the drug and sustained release continued until day 28. After the burst release around day 30 to 34, further release was not detected. The result is expressed as the mean of two experiments; bars, S.D. C) Release from the drug-overloaded sheet. When the drug concentration was increased 3-fold when co-polymerized to PLGA, the sheet released the drug much faster than the ordinary sheet. Most of the drug was released by day 8 and further release was not prominent after day 10. The result is expressed as the mean of two experiments; bars, S.D.*

quantified at the 480-nm wavelength. The PLGA sheet containing doxorubicin was left under physiological conditions and the total amounts of doxorubicin released were measured (Figure 1B). Release started from day 1 and gradually increased until day 24. Subsequently, the release was abruptly increased and continued until day 34. Thirty-four days after the experiment, the release reached a peak.

The released amount was followed up until day 178, however further release was not detected in the experiment (data not shown). The pattern of slow release from the sheet might derive from the proportions of doxorubicin and PLGA. When the load of doxorubicin was increased in the sheet, a three-fold higher drug discharge occurred at an earlier stage of the experiment (Figure 1C). The drug burst
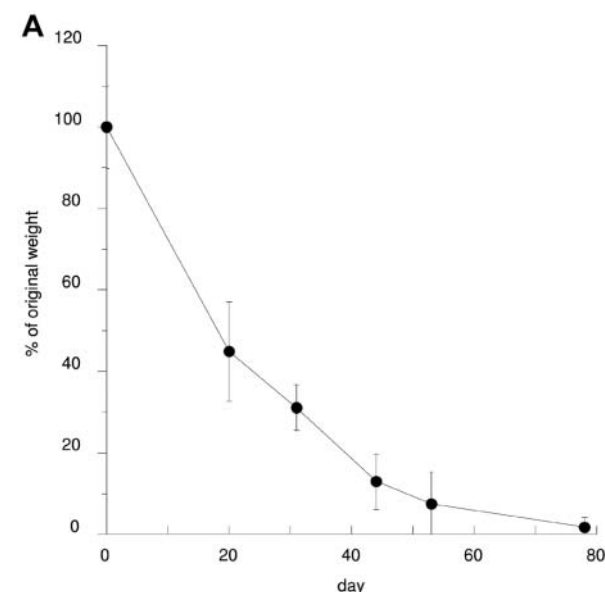
started from the day of the experiment and most of the doxorubicin was released by day 8. Unlike the previous result, sustained release was not detected in this drug-enriched sheet. The sheet did not retain doxorubicin after 12 days of experiments.

Biodegradation of the sheet in mice. Since deliberate release of the drug from the sheet was demonstrated *in vitro*, the biodegradability of the sheet was examined next. After implantation of the sheet into the left flank of the mice, changes in the dry-weight of the sheet were measured and recorded chronologically. The sheet degraded according to the passage of time. Degradation rapidly progressed in the initial stage and continued until day 78. The sheet was ultimately absorbed. It took more than 80 days to disappear and further changes in weight could not be determined. During the process, the doxorubicin sheet was assimilated and other than pigmentation in the adjacent area, caused

Figure 2. *Biodegradability of the sheet in vivo. A) The dry-weight of the implanted sheet was measured and biodegradability was expressed as a percentage of the original weight. The sheet degraded according to the passage of time. There was a rapid decrease in volume from the start of the experiment, followed by gradual degradation. More than 78 days were required for complete absorption. The result is expressed as the mean of five animals at each time point; bars, S.D. B) Biodegradability of the subcutaneously implanted sheet. The picture shows the sheets at 52 days after implantation. The sheet was degraded, but still visible with a change in the color of the surrounding subcutaneous tissue. Pigmentation of tissue occurred in the contact area of the sheet.*
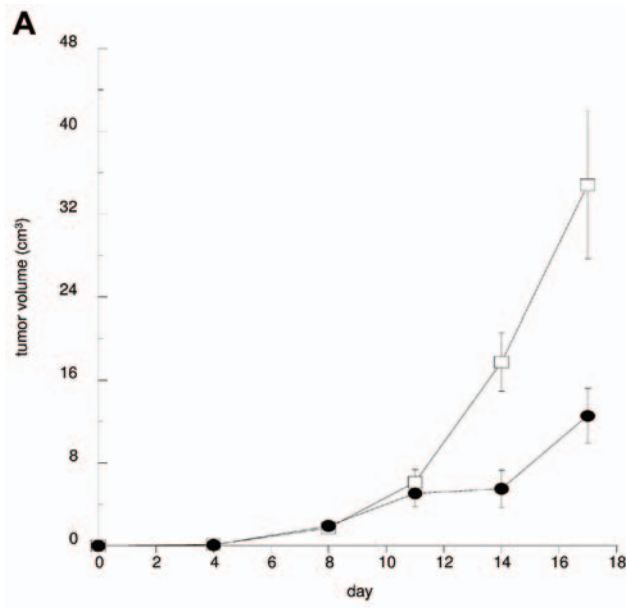
neither inflammation nor substantial necrosis in the surrounding tissue (Figure 2A, B).

Effect of the released doxorubicin on the established tumor. The slow-release character and biodegradability of the sheet enables potential application of the sheet for tumor treatment *in vivo*. In the final examination, the sheet was used for the treatment of subcutaneously implanted RT2 syngeneic malignant glioma tumor cells. After growth, the tumor was covered with a doxorubicin sheet and the subsequent growth was measured. Tumors treated with a mock sheet increased in size exponentially (Figure 3). In contrast, growth of the tumor was inhibited in rats treated with the doxorubicin sheet. On the 17th day of the experiment, the tumor volume reached more than 30 cm^3 and the rats started to die in the control group, whereas the group treated with the doxorubicin sheet exhibited a smaller tumor size. There were inter-group differences in volumes
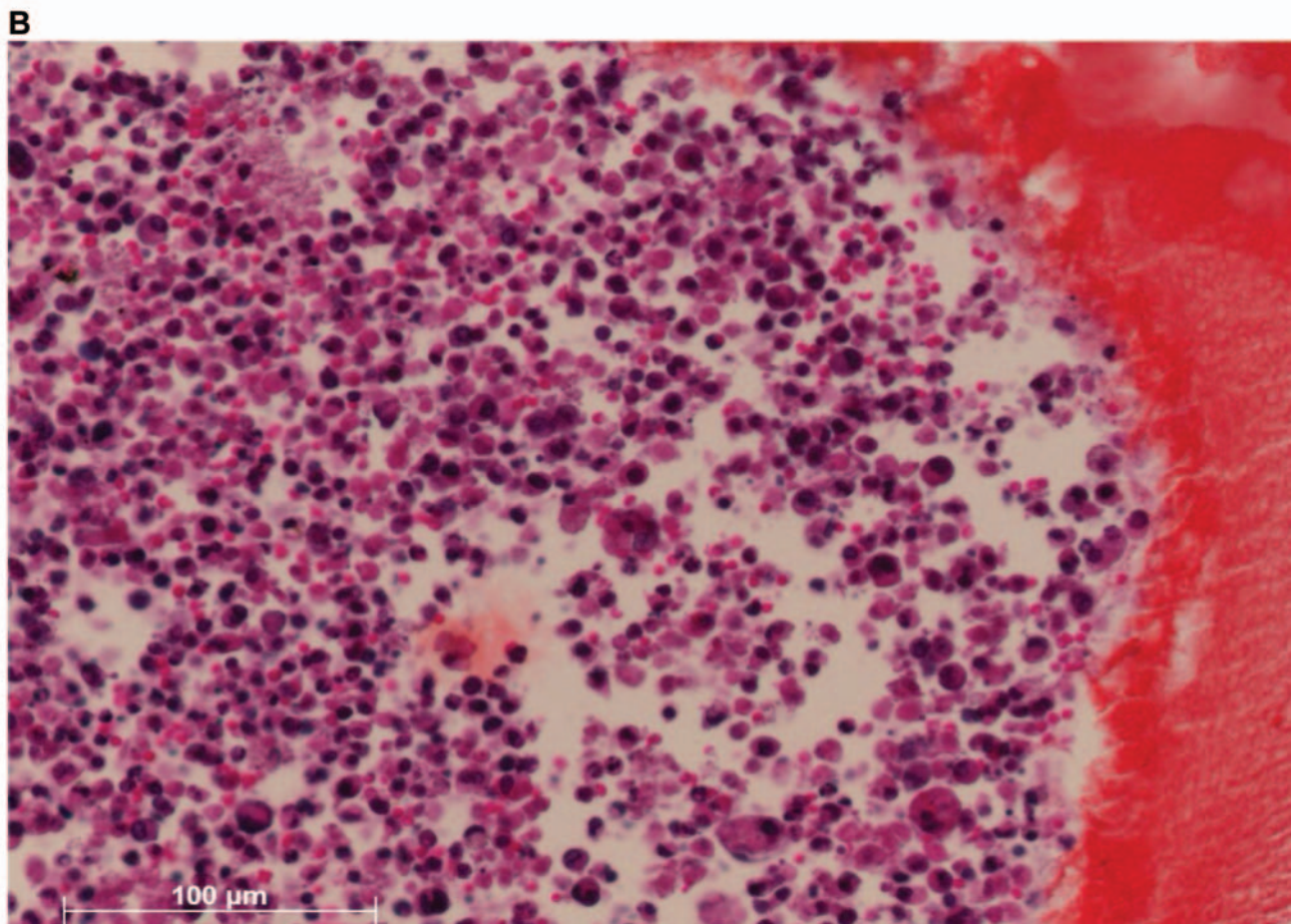
Figure 3. *Tumor growth inhibition by the sheet. A) After glioma cells were implanted, the tumor nodule was treated to the sheet. While tumors in control animals grew prosperously, treatment inhibited the expansion of the tumor. Mock sheet treatment (□); doxorubicin sheet treatment (●). There were differences on day 14 (p=0.064) and day 17 (p=0.019). The result was demonstrated as a mean of five animals in each group; bars, S.D. B) Histology of tumor cells with the sheet (on day 17, hematoxylin-eosin staining). Tumor tissue or cells (left) adjoining the sheet (right) were necrotic with erythrocytes.*
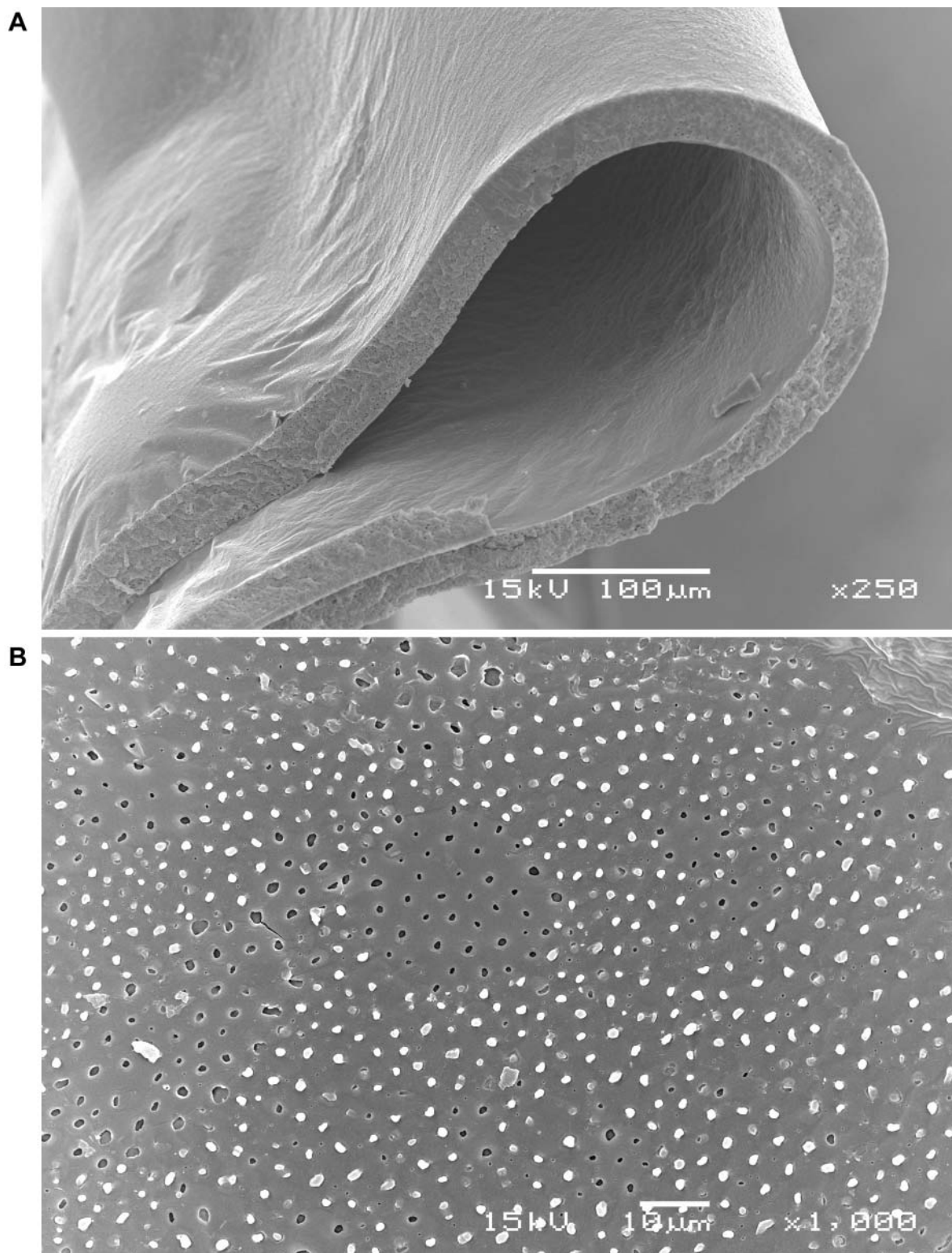
Figure 4. *Ultrastructure of doxorubicin sheet by electron microscopy. A-C) Pictures taken by scanning electron microscope, D) By transmission electron microscope. A) Overview: the sheet had a flexible texture with a thickness of 10 µm. B) Surface: the surface consisted of amorphous material with small holes. Grains of the drug resided in these small holes with a diameter of 0.5 to 3 µm. C) Vertical section (ethanol-cracked surface): after fixation, the sample was ethanol-cracked in liquid nitrogen. Cross-section disclosed the porous structure of the membrane sheet. D) Cross-section of the sheet: the drug was encircled by an amorphous electro-density substrate. Direct magnification, x15000.*
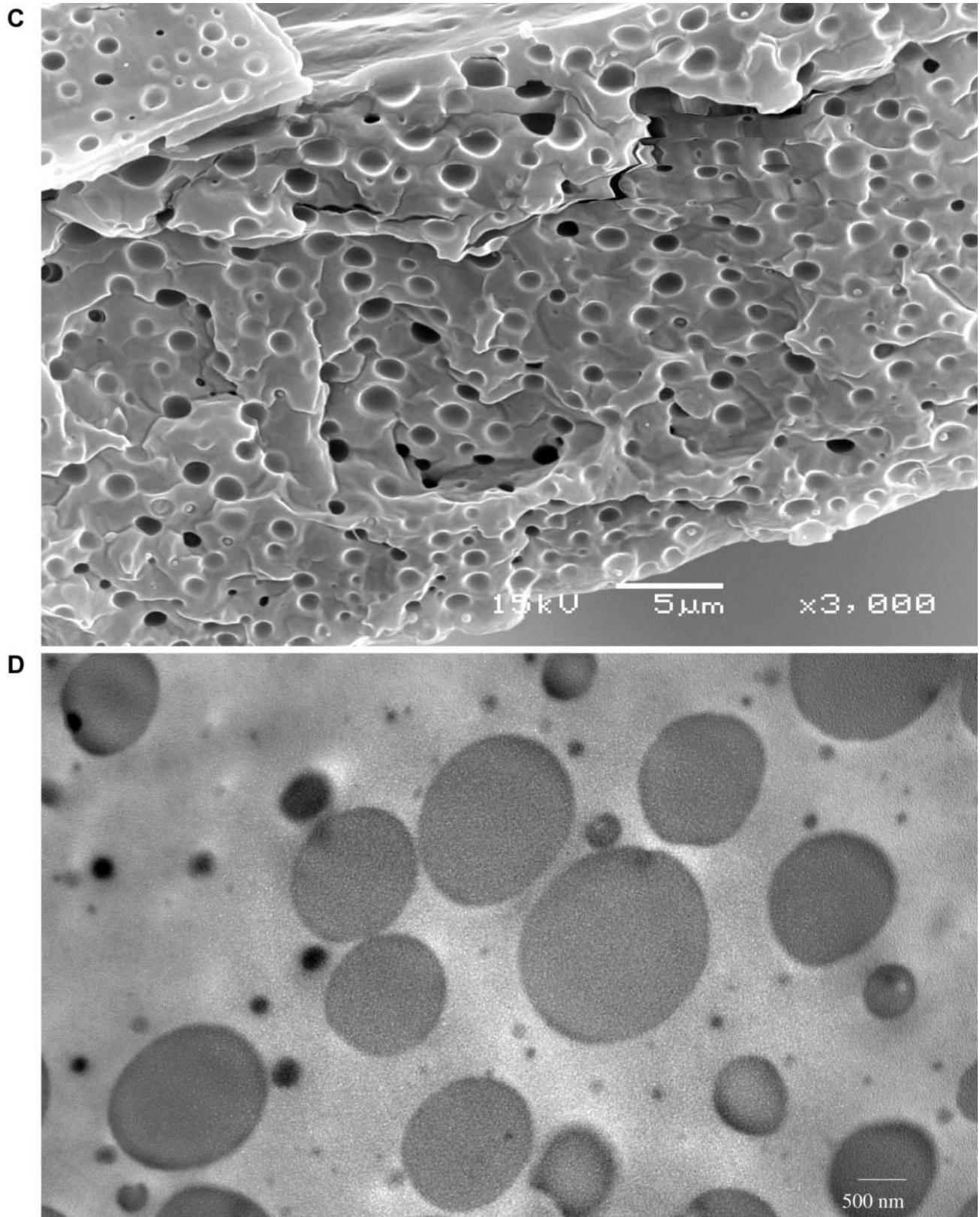
Figure 4. *continued*

on days 14 and 17 ($p=0.064$ and 0.019, respectively). The sizes of tumors treated with the doxorubicin sheet were comparable to those of animals treated by direct injection with a 4-times higher total dose (on day 14: injection 4.88 ± 2.33 cm^3 vs. sheet 5.50 ± 1.81 cm^3, on day 17: 14.80 ± 6.62 cm^3 vs. 12.56 ± 2.65 cm^3).

Morphological studies of the sheet. The sheet's ability to confer toxicity to the target tumor by releasing the drug was confirmed. To further investigate the material, the sheet was examined by electronmicroscopy. The sheet had a thickness of 10 μm and was flexible (Figure 4A). The surface of the sheet consisted of an amorphous structure with small cavities having a diameter of 0.5 to 3 μm. A grain, presumably of drug, was held in each cavity and some of these protruded to the surface. Some of the cavities were empty, but this may have been due to elution of the drug during preparation of the specimen (Figure 4B). An ethanol-cracked, vertical section revealed the spongy, cheese-like structure of the sheet. Most of the cavity was hollow due to the same reason as above, but the drug is visible in the cavities through a small exit (Figure 4C). This finding was confirmed by transmission electronmicroscopy (Figure 4D). The structure of the sheet may be responsible for sustained release of the drug.

Discussion

In this study, a doxorubicin-loaded poly (D, L-lactide-co-glycolide) membrane was developed and drug release from the membrane, biodegradation and efficacy on implanted glioma cells were examined.

As a scaffold for drug polymerization, PLGA was chosen. Similar to other polymers (14), PLGA has been used, not only as biodegradable polyester elastomers in tissue engineering (15), but also as a carrier of drugs, antigens, or genes either by itself or in combination with other appropriate materials. Owing to its safety, performance, cost and ease-of-use, this material was especially useful as a drug delivery tool for anticancer drugs. Micro- or nano-particles of PLGA conjugates include paclitaxel (16-20), doxorubicin (21-23), floxuridine (24), cystatins (25), camptothetin (26), 5-fluorouracil (27, 28), oxaliplatin (29), methotrexate (30) and cisplatin (31). In addition to the anticancer agents, tumor antigen (32, 33), photodynamic (34-37) or radiosensitizer (38, 39), genes (40-42) or DNA decoys (43), anti-angiogenic agents (44, 45), usnic acid (46), interferons (47), immunotoxin (48), all-trans retinoic acid (49), hormones (42, 50) and other compounds have been conjugated to PLGA for the treatment of malignant diseases.

Nano- or micro-particles of PLGA have drug delivery advantages, such as achievement of a higher concentration in the target tissue, sustained release and a longer circulation time in plasma as well as lower toxicity. However, from the stand-point of brain tumor therapy, especially considering the prevention of recurrence, there is an advantage of local therapy with an implantable drug-conjugated device, even though diffusion of nanoparticles is relatively limited to the vicinity of the implantation site (27). Accordingly, a wafer with BCNU was successfully developed (7, 8, 51). In other solid tumors, local treatment with PLGA polymers with paclitaxel and vinca alkaloid were developed and tested in clinical pilot trials (52, 53).

We chose doxorubicin for co-polymerization to PLGA. This drug has a long history and has been used widely for the treatment of malignancies, including leukemias, lymphomas and many solid tumors, including brain tumors. Accordingly, its pharmacokinetics are well known. From the aspect of safety, the drug can be administered intrathecally with few serious adverse effects (54, 55). This might compromise safety if leakage of the drug occurs into the cerebrospinal fluid. Moreover, resistance to alkylating agent due mainly to overexpression of MGMT generally does not demonstrate cross-resistance to doxorubicin, which blocks DNA and RNA synthesis by inhibiting topoisomerase II. The sheet might be especially useful for patients with recurrent drug-resistant gliomas initially treated by alkylating agents.

Local therapies are key options for the treatment of brain tumors. BCNU-loaded wafers and other implantable nano- and micro-particles are the materials of first choice. It is preferable to increase the number of effective devices or drugs for local treatment. Since our PLGA-based sheet is implantable, easy to prepare, wholly degradable and displays a sustained-release property, it may play a role in the treatment for malignant brain tumors as a local therapy device.

Acknowledgements

References

1 Ohgaki H and Kleihues P: Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol *64*: 479-489, 2005.

2 Barrie M, Couprie C, Dufour H, Figarella-Branger D, Muracciole X, Hoang-Xuan K, Braguer D, Martin PM, Peragut JC, Grisoli F and Chinot O: Temozolomide in combination with BCNU before and after radiotherapy in patients with inoperable newly diagnosed glioblastoma multiforme. Ann Oncol *16*: 1177-1184, 2005.

3 Cohen MH, Johnson JR and Pazdur R: Food and Drug Administration Drug approval summary: temozolomide plus radiation therapy for the treatment of newly diagnosed glioblastoma multiforme. Clin Cancer Res *11*: 6767-6771, 2005.

4 Yoshii Y, Maki Y, Tsuboi K, Tomono Y, Nakagawa K and Hoshino T: Estimation of growth fraction with bromodeoxyuridine in human central nervous system tumors. J Neurosurg 65: 659-663, 1986.

5 Hamstra DA, Moffat BA, Hall DE, Young JM, Desmond TJ, Carter J, Pietronigro D, Frey KA, Rehemtulla A and Ross BD: Intratumoral injection of BCNU in ethanol (DTI-015) results in enhanced delivery to tumor – a pharmacokinetic study. J Neurooncol 73: 225-238, 2005.

6 Yimam MA, Bui T and Ho RJ: Effects of lipid association on lomustine (CCNU) administered intracerebrally to syngeneic 36B-10 rat brain tumors. Cancer Lett, 2006.

7 Seong H, An TK, Khang G, Choi SU, Lee CO and Lee HB: BCNU-loaded poly(D, L-lactide-co-glycolide) wafer and antitumor activity against XF-498 human CNS tumor cells *in vitro*. Int J Pharm 251: 1-12, 2003.

8 Lee JS, An TK, Chae GS, Jeong JK, Cho SH, Lee HB and Khang G: Evaluation of *in vitro* and *in vivo* antitumor activity of BCNU-loaded PLGA wafer against 9L gliosarcoma. Eur J Pharm Biopharm 59: 169-175, 2005.

9 Westphal M, Hilt DC, Bortey E, Delavault P, Olivares R, Warnke PC, Whittle IR, Jaaskelainen J and Ram Z: A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. Neuro-oncol 5: 79-88, 2003.

10 Bobola MS, Silber JR, Ellenbogen RG, Geyer JR, Blank A and Goff RD: O6-methylguanine-DNA methyltransferase, O_6-benzylguanine, and resistance to clinical alkylators in pediatric primary brain tumor cell lines. Clin Cancer Res 11: 2747-2755, 2005.

11 Hermisson M, Klumpp A, Wick W, Wischhusen J, Nagel G, Roos W, Kaina B and Weller M: O_6-methylguanine DNA methyltransferase and p53 status predict temozolomide sensitivity in human malignant glioma cells. J Neurochem 96: 766-776, 2006.

12 Manome Y, Watanabe M, Futaki K, Ishiguro H, Iwagami S, Noda K, Dobashi H, Ochiai Y, Ohara Y, Sanuki K, Kunieda T and Ohno T: Development of a syngenic brain-tumor model resistant to chloroethyl-nitrosourea using a methylguanine DNA methyltransferase cDNA. Anticancer Res 19: 5313-5318, 1999.

13 Manome Y, Yoshinaga H, Watanabe M and Ohno T: Adenoviral transfer of antisenses or ribozyme to O_6-methylguanine-DNA methyltransferase mRNA in brain-tumor model resistant to chloroethyl-nitrosourea. Anticancer Res 22: 2029-2036, 2002.

14 Lesniak MS, Upadhyay U, Goodwin R, Tyler B and Brem H: Local delivery of doxorubicin for the treatment of malignant brain tumors in rats. Anticancer Res 25: 3825-3831, 2005.

15 Webb AR, Yang J and Ameer GA: Biodegradable polyester elastomers in tissue engineering. Expert Opin Biol Ther 4: 801-812, 2004.

16 Fonseca C, Simoes S and Gaspar R: Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and *in vitro* anti-tumoral activity. J Control Release 83: 273-286, 2002.

17 Kang BK, Chon SK, Kim SH, Jeong S, Kim MS, Cho SH, Lee HB and Khang G: Controlled release of paclitaxel from microemulsion containing PLGA and evaluation of anti-tumor activity *in vitro* and *in vivo*. Int J Pharm 286: 147-156, 2004.

18 Mo Y and Lim LY: Paclitaxel-loaded PLGA nanoparticles: potentiation of anticancer activity by surface conjugation with wheat germ agglutinin. J Control Release 108: 244-262, 2005.

19 Mo Y and Lim LY: Preparation and *in vitro* anticancer activity of wheat germ agglutinin (WGA)-conjugated PLGA nanoparticles loaded with paclitaxel and isopropyl myristate. J Control Release. 107: 30-42, 2005.

20 Sahoo SK and Labhasetwar V: Enhanced antiproliferative activity of transferrin-conjugated paclitaxel-loaded nanoparticles is mediated *via* sustained intracellular drug retention. Mol Pharm 2: 373-383, 2005.

21 Lin R, Shi Ng L and Wang CH: *In vitro* study of anticancer drug doxorubicin in PLGA-based microparticles. Biomaterials 26: 4476-4485, 2005.

22 Yoo HS and Park TG: Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA-PEG block copolymer. J Control Release 70: 63-70, 2001.

23 Mallery SR, Pei P, Kang J, Ness GM, Ortiz R, Touhalisky JE and Schwendeman SP: Controlled-release of doxorubicin from poly(lactide-co-glycolide) microspheres significantly enhances cytotoxicity against cultured AIDS-related Kaposi's sarcoma cells. Anticancer Res 20: 2817-2825, 2000.

24 Inoue K, Onishi H, Kato Y, Michiura T, Nakai K, Sato M, Yamamichi K, Machida Y and Nakane Y: Comparison of intraperitoneal continuous infusion of floxuridine and bolus administration in a peritoneal gastric cancer xenograft model. Cancer Chemother Pharmacol 53: 415-422, 2004.

25 Cegnar M, Premzl A, Zavasnik-Bergant V, Kristl J and Kos J: Poly(lactide-co-glycolide) nanoparticles as a carrier system for delivering cysteine protease inhibitor cystatin into tumor cells. Exp Cell Res 301: 223-231, 2004.

26 Tong W, Wang L and D'Souza MJ: Evaluation of PLGA microspheres as delivery system for antitumor agent-camptothecin. Drug Dev Ind Pharm 29: 745-756, 2003.

27 Roullin VG, Deverre JR, Lemaire L, Hindre F, Venier-Julienne MC, Vienet R and Benoit JP: Anti-cancer drug diffusion within living rat brain tissue: an experimental study using [^{3}H](6)-5-fluorouracil-loaded PLGA microspheres. Eur J Pharm Biopharm 53: 293-299, 2002.

28 Hagiwara A, Sakakura C, Shirasu M, Yamasaki J, Togawa T, Takahashi T, Muranishi S, Hyon S and Ikada Y: Therapeutic effects of 5-fluorouracil microspheres on peritoneal carcinomatosis induced by Colon 26 or B-16 melanoma in mice. Anticancer Drugs 9: 287-289, 1998.

29 Lagarce F, Cruaud O, Deuschel C, Bayssas M, Griffon-Etienne G and Benoit J: Oxaliplatin loaded PLAGA microspheres: design of specific release profiles. Int J Pharm 242: 243-246, 2002.

30 Singh UV and Udupa N: *In vitro* characterization of methotrexate loaded poly(lactic-co-glycolic) acid microspheres and antitumor efficacy in Sarcoma-180 mice bearing tumor. Pharm Acta Helv 72: 165-173, 1997.

31 Kumagai S, Sugiyama T, Nishida T, Ushijima K and Yakushiji M: Improvement of intraperitoneal chemotherapy for rat ovarian cancer using cisplatin-containing microspheres. Jpn J Cancer Res 87: 412-417, 1996.

32 Waeckerle-Men Y and Groettrup M: PLGA microspheres for improved antigen delivery to dendritic cells as cellular vaccines. Adv Drug Deliv Rev 57: 475-482, 2005.

33 Waeckerle-Men Y, Scandella E, Uetz-Von Allmen E, Ludewig B, Gillessen S, Merkle HP, Gander B and Groettrup M: Phenotype and functional analysis of human monocyte-derived dendritic cells loaded with biodegradable poly(lactide-co-glycolide) microspheres for immunotherapy. J Immunol Methods 287: 109-124, 2004.

34 McCarthy JR, Perez JM, Bruckner C and Weissleder R: Polymeric nanoparticle preparation that eradicates tumors. Nano Lett *5*: 2552-2556, 2005.

35 Vargas A, Pegaz B, Debefve E, Konan-Kouakou Y, Lange N, Ballini JP, van den Bergh H, Gurny R and Delie F: Improved photodynamic activity of porphyrin loaded into nanoparticles: an *in vivo* evaluation using chick embryos. Int J Pharm *286*: 131-145, 2004.

36 Konan YN, Berton M, Gurny R and Allemann E: Enhanced photodynamic activity of meso-tetra(4-hydroxyphenyl)porphyrin by incorporation into sub-200 nm nanoparticles. Eur J Pharm Sci *18*: 241-249, 2003.

37 Konan YN, Chevallier J, Gurny R and Allemann E: Encapsulation of p-THPP into nanoparticles: cellular uptake, subcellular localization and effect of serum on photodynamic activity. Photochem Photobiol *77*: 638-644, 2003.

38 Geze A, Venier-Julienne MC, Saulnier P, Varlet P, Daumas-Duport C, Devauchelle P and Benoit JP: Modulated release of IdUrd from poly (D,L-lactide-co-glycolide) microspheres by addition of poly (D,L-lactide) oligomers. J Control Release *58*: 311-322, 1999.

39 Reza MS and Whateley TL: Iodo-2'-deoxyuridine (IUdR) and 125IUdR loaded biodegradable microspheres for controlled delivery to the brain. J Microencapsul *15*: 789-801, 1998.

40 Prabha S and Labhasetwar V: Critical determinants in PLGA/PLA nanoparticle-mediated gene expression. Pharm Res *21*: 354-364, 2004.

41 Jilek S, Ulrich M, Merkle HP and Walter E: Composition and surface charge of DNA-loaded microparticles determine maturation and cytokine secretion in human dendritic cells. Pharm Res *21*: 1240-1247, 2004.

42 Panyam J, Zhou WZ, Prabha S, Sahoo SK and Labhasetwar V: Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. Faseb J *16*: 1217-1226, 2002.

43 Gill JS, Zhu X, Moore MJ, Lu L, Yaszemski MJ and Windebank AJ: Effects of NFkappaB decoy oligonucleotides released from biodegradable polymer microparticles on a glioblastoma cell line. Biomaterials *23*: 2773-2781, 2002.

44 Benny O, Duvshani-Eshet M, Cargioli T, Bello L, Bikfalvi A, Carroll RS and Machluf M: Continuous delivery of endogenous inhibitors from poly(lactic-co-glycolic acid) polymeric microspheres inhibits glioma tumor growth. Clin Cancer Res *11*: 768-776, 2005.

45 Bandi N, Ayalasomayajula SP, Dhanda DS, Iwakawa J, Cheng PW and Kompella UB: Intratracheal budesonide-poly(lactide-co-glycolide) microparticles reduce oxidative stress, VEGF expression, and vascular leakage in a benzo(a)pyrene-fed mouse model. J Pharm Pharmacol *57*: 851-860, 2005.

46 Ribeiro-Costa RM, Alves AJ, Santos NP, Nascimento SC, Goncalves EC, Silva NH, Honda NK and Santos-Magalhaes NS: *In vitro* and *in vivo* properties of usnic acid encapsulated into PLGA-microspheres. J Microencapsul *21*: 371-384, 2004.

47 Sanchez A, Tobio M, Gonzalez L, Fabra A and Alonso MJ: Biodegradable micro- and nanoparticles as long-term delivery vehicles for interferon-alpha. Eur J Pharm Sci *18*: 221-229, 2003.

48 Ferdous AJ, Stembridge NY and Singh M: Role of monensin PLGA polymer nanoparticles and liposomes as potentiator of ricin A immunotoxins *in vitro*. J Control Release *50*: 71-78, 1998.

49 Jeong YI, Song JG, Kang SS, Ryu HH, Lee YH, Choi C, Shin BA, Kim KK, Ahn KY and Jung S: Preparation of poly(DL-lactide-co-glycolide) microspheres encapsulating all-trans retinoic acid. Int J Pharm *259*: 79-91, 2003.

50 Labrie F, Li S, Belanger A, Cote J, Merand Y and Lepage M: Controlled release low dose medroxyprogesterone acetate (MPA) inhibits the development of mammary tumors induced by dimethyl-benz(a) anthracene in the rat. Breast Cancer Res Treat *26*: 253-265, 1993.

51 Chae GS, Lee JS, Kim SH, Seo KS, Kim MS, Lee HB and Khang G: Enhancement of the stability of BCNU using self-emulsifying drug delivery systems (SEDDS) and *in vitro* antitumor activity of self-emulsified BCNU-loaded PLGA wafer. Int J Pharm 301: 6-14, 2005.

52 Rohr UD, Oberhoff C, Markmann S, Gerber B, Scheulen M and Schindler AE: The safety of synthetic paclitaxel by intralesional delivery with OncoGeltrade mark into skin breast cancer metastases: method and results of a clinical pilot trial. Arch Gynecol Obstet 1-7, 2005.

53 Fournier C, Hecquet B, Bouffard P, Vert M, Caty A, Vilain MO, Vanseymortier L, Merle S, Krikorian A, Lefebvre JL *et al*: Experimental studies and preliminary clinical trial of vinorelbine-loaded polymeric bioresorbable implants for the local treatment of solid tumors. Cancer Res *51*: 5384-5391, 1991.

54 Jordan B, Pasquier Y and Schnider A: Neurological improvement and rehabilitation potential following toxic myelopathy due to intrathecal injection of doxorubicin. Spinal Cord *42*: 371-373, 2004.

55 Arico M, Nespoli L, Porta F, Caselli D, Raiteri E and Burgio GR: Severe acute encephalopathy following inadvertent intrathecal doxorubicin administration. Med Pediatr Oncol *18*: 261-263, 1990.